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Bacteriophage T3 and Bacteriophage T7 Virus-Host Cell Interactions

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INTRODUCTION

The recognition in the 1940s that bacteriophages could help to elucidate the molecular basis of heredity (49) has initiated exceedingly successful development. Phages, and viruses in general, are relatively simple systems and much easier to analyze than are complex living systems. The study of bacteriophages has contributed much to the science of virology, since most phenomena originally observed in phage-infected bacteria were later on also discovered in

the interactions between eucaryote viruses and their host cells. The substantial technical advance during the past years has made it possible to explore molecular events in complex eucaryote cells and has established the intrinsic equivalence of pro- and eucaryote molecular genetics as well as a number of differences between them. The accessibility of eucaryote systems to molecular biological research does not, however, warrant the conclusion that the days of bacteriophage are over. Bacteriophages remain important models for virus-cell interaction: current research on bacteriophages is devoted to such problems as the mechanisms of virus-effected killing of cells, the integration and excision of viral genes in and from host genomes, deoxyribonucleic acid (DNA) modification and restriction, the reconstitution of the whole process of DNA replication in vitro, and solving the puzzle of temporal gene expression; these topics are of supreme interest for animal virology, too. In addition, bacteriophages have gained great scientific and economic prominence as vehicles for experimental gene transfer.

Bacteriophages T3 and T7 belong to the classical T series of bacteriophages (94). Not only the traditional *Escherichia coli* laboratory strains but also certain strains of *Shigella sonnei* (140, 164), *Salmonella typhimurium* (43), *Klebsiella pneumoniae* (379, 424), and *Pasteurella* (165) are permissive hosts. However, all essential knowledge about T3 and T7 virus-host interactions and the molecular biology of T3 and T7 was acquired in the *E. coli* system.

In writing this review it was a great advantage that we could rely on the excellent reviews of Hausmann (164, 165), McCorquodale (306), and Studier (484, 489), and, while concentrating on new developments, we shall refer to the 1976 review of Hausmann (165) for most of the work published up to that time. Faced with an avalanche of new papers, we are aware of the fact that the wealth of information can no longer be adequately presented in a condensed review; there is by now enough material to fill a book exclusively dedicated to these phages, a book like the modern classic *The Bacteriophage Lambda* (181). Therefore, we were forced to select topics for thorough treatment at the expense of others which were only briefly summarized.

GENERAL PROPERTIES

The Viruses and Their Growth Cycle

The principal features of the T3 and T7 phages' structure and growth cycle have been thoroughly reviewed (164, 165, 306, 484, 489) and are briefly summarized here. The virion consists of a polyhedral nucleocapsid 50 nm in diameter to which a simple, noncontractile tail 20 nm long is attached which is capable of adsorbing to bacterial cells. The genome ejected through this tail is a single molecule of linear double-stranded DNA with a molecular weight of about 25×10^6, equivalent to approximately 40,000 base pairs. (The molecular weight of T7 DNA has recently been determined with greater accuracy to be 26.4×10^6 to 26.5×10^6 [76, 482]. According to a recent estimation, T3 DNA contains 38.74 kilobases [17], which is equivalent to a molecular weight of 25.6×10^6.) The DNA consists of the usual four nucleotides, the guanine plus cytosine content being similar to that of the *E. coli* host cell. The DNA is terminally redundant, but not circularly permuted, and codes for about 30 proteins. Gene expression of the phages is relatively simple, thanks to the exclusive transcription of the parental genome(s) and only of the heavy DNA strand thereof, as well as to the strictly sequential reading of the genes in the order in which they are arranged on the phage chromosome.

Phage development involves a number of regulated steps effecting shutoff of host and early phage functions, the degradation of the host genome into nucleotides, and their highly economical reutilization in the rapid synthesis of phage progeny DNA.

On infection of typical permissive *E. coli* strains under standard conditions, lysis begins at 12 to 15 min (37°C) or 20 to 25 min (30°C) and an average of 200 progeny phage are released per cell.

T3 and T7 are closely related, and their DNA molecules hybridize extensively; however, there are several functionally important nonhomologous regions. One of the salient differences between the two phages is the coding of an adenosylmethionine hydrolase (SAMase) by gene 0.3 of T3, but not by T7 (see Adenosylmethionine Hydrolase). Another nonhomologous region lies within gene 1, affecting the molecular weight and the template specificity of the ribonucleic acid (RNA) polymerase (RNA nucleotidyltransferase) (see Transcription). Differences between the two phages also show up in gene 17, where they determine the nonidentical antigenicities and adsorption specificities of the tail fiber proteins (see Adsorption and DNA Injection).

Restriction Analysis of Phage Deoxyribonucleic Acid (DNA)

The utility of sequence-specific restriction endonucleases for physical mapping and sequence analysis of genomes and for gene cloning (272, 327, 328, 394, 395, 466) has had its impact also

on the elucidation of the structure and function of the T3 and T7 phage genomes. After the initial *Hin*dII digestion performed by Kelly and Smith (220), and extension of this work (201, 259), several other restriction endonucleases were introduced into the analysis of T7 DNA (50, 147, 148, 275, 276, 300, 302, 307, 355, 494). The most extensive compilation of restriction analyses of the T7 genome was published by Rosenberg et al. (405). T7 DNA bears no recognition sites for the enzymes *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*I, *Sal*I, *Sma*I, *Sst*I, *Sst*II, and *Xho*I; one to six recognition sites exist for *Ava*I, *Bal*I, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Bst*NI, *Cla*I, *Dpn*II, *Kpn*I, *Pvu*II, *Xba*I, *Xho*II (405), and *Eco*RII (W. Wackernagel, personal communication); and the DNA is cut at several sites by *Acc*I, *Alu*I, *Ava*II, *Hae*II, *Hae*III, *Hga*I, *Hgi*AI, *Hha*I, *Hin*dII, *Hin*fI, *Hpa*I, *Hpa*II, *Hph*I, *Mbo*II, *Mnl*I, *Sau*96I, *Taq*I, *Tha*I (405), and *Bsu*R (148). After initial experiments with *Hap*II, *Hga*I, *Hin*HI (502), and *Hpa*I (490), a detailed restriction map of T3 DNA has now been established by Bailey et al. (17). T3 DNA possesses no recognition sites for *Bam*HI, *Bgl*I, *Eco*RI, *Pst*I, *Pvu*II, *Sal*I, *Sma*I, *Sst*II, and *Xho*I; one to six sites for *Ava*I, *Bgl*II, *Hin*dIII, *Kpn*I, and *Xba*I; and several sites for *Hpa*I, *Mbo*I (17), *Hap*II, and *Hin*HI (502). An alignment of the T3 and T7 restriction maps reveals substantial differences (17, 405). Since differences also exist between laboratory strains of the same phage species, Studier (490) recommends a comparison of restriction patterns of phage from various laboratories in order to test their identity.

Physical maps of the early T7 genome region were established (147, 276, 300, 494), and certain genome fragments were isolated to study their interaction with *E. coli* and T7 RNA polymerase and for hybridization mapping of phage messenger RNA (mRNA) (200, 212, 217, 300, 302). In addition, restriction fragments were used for sequence analysis of T7 promoters (304, 336, 338, 404, 456).

Cloning of DNA fragments with the aid of plasmid or virus vehicles has opened new perspectives for the investigation of the molecular biology of T3 and T7. Campbell et al. (50) cloned some late and early T7 genes on the plasmid pMB9; in addition, the origin of DNA replication (51, 356, 423a) and promoter regions were cloned (346, 347, 435). Recently, the T7 gene 0.3 (*ocr*$^+$) has been inserted into a bacteriophage lambda vector (363). Tsujimoto and co-workers took advantage of the fact that T7 wild-type DNA lacks *Eco*RI recognition sites to study its recombination with DNA of an *Eco*RI-sensitive T7 mutant (341, 508–510).

T7 DNA has a lasting place in the history of science, as it was the first target for a sequence-specific restriction endonuclease (*Hin*dII) tested in 1970 by Smith and co-workers (220, 467). From then on, type II restriction enzymes were used as tools for molecular biology (272, 327, 394, 466). It is curious that the interaction of *Hin*dII with T7 DNA is of no significance in vivo, since T7 does not even adsorb to the *Hin*dII-producing bacterium *Haemophilus influenzae* R_d (D. H. Krüger and M. Hartmann, unpublished data).

EARLY VIRUS-CELL INTERACTIONS

Adsorption and DNA Injection

The *Host range* of a virus is defined as comprising those hosts which permit a productive viral growth cycle. We shall use this term in the more restricted sense of cells to which the virus can adsorb. The ability of the virus to adsorb and the presence or absence of virus receptors on a cell surface constitute the first level of virus-host interaction on which the establishment of infection is decided. The physical chemistry of virus adsorption has been largely neglected by current research, so there is little progress to report. The receptors for T3 and T7 consist of lipopolysaccharide areas in the outer membrane of the cell wall (38, 271, 373, 521). The tail fiber protein encoded by gene 17 is the main determinant of phage adsorption specificity (206, 207; O. G. Issinger, Ph.D. thesis, University of Freiburg, Freiburg, Federal Republic of Germany, 1973).

T3 and T7 genes 17 display a high degree of homology (89), which explains the related host ranges of these phages. The described host range of T7 is, however, broader than that of T3, since T7-resistant strains of *E. coli* are also resistant to T3, but not necessarily vice versa (94, 148, 159). Recent publications of Studier (490) and Krüger et al. (246) describe the resistance of *E. coli* K-12 Wisconsin (W) strains to T3 as opposed to their permissiveness for T7 infection; T3 host range mutants ($T3h_W$) were isolated which adsorb to *E. coli* W as well as T7 (246). Another distinction between T3 and T7 is that the latter adsorbs with a higher rate to *E. coli* B cells (171; H. Beier, Ph.D. thesis, University of Freiburg, Freiburg, Federal Republic of Germany, 1973).

Corresponding to the similarity of the phage receptors, antisera to T3 and T7 are cross-reactive (3, 172, 206, 246; Issinger, Ph.D. thesis). The adsorption properties and serologies of T3 and T7 seem to be multifactorially determined, since the $T3h_W$ mutants adsorb to *E. coli* W, like T7, but retain their typical T3 adsorption pattern on different *E. coli* B strains (246, 249). The $T3h_W$

mutation also alters the serological specificity of the phage, rendering it intermediate between those of T3 and T7 (246).

Triggered by cell-virus receptor interaction, the phage particle injects its DNA into the cell, beginning with the genetic left end (357, 417), the transcription of which appears to be the precondition for the complete injection of the genome (544).

Nonclassical Modification of Viruses

It has been known for a long time that the ability of a phage to grow on a certain host strain can be dependent on a modification of the phage received in the previous host cell (277). When the modification is disadvantageous, phage growth on the new host is restricted. The criterion distinguishing in vivo modification and restriction (M/R) from mutational effects is the demonstration of reversible, host-dependent changes in the efficiency of plating of the phage (37, 277). Up to now, host-controlled M/R of bacteriophages has always been connected to modification versus cleavage of phage DNA (see Interactions with the DNA Modification and Restriction System of the Host: Functions of Gene 0.3). Krüger and co-workers (247–250) proved that, in addition, a totally different mechanism of host-dependent M/R exists which influences the adsorption ability of the phages and is caused by protein modification. In contrast to the "classical" M/R of DNA, this phenomenon was termed "nonclassical" M/R (247–250).

Such a nonclassical M/R was observed when T7 and SAMase-negative T3 derivatives were passaged on different *E. coli* B strains (250). The efficiency of plating of the phage varies from 0.01 to 1.0, dependent on the host strain on which the phage were last grown. These genetically unaltered phage carrying different nonclassical modifications also differ antigenically (K. K. Gachechiladze, D. H. Krüger, N. S. Balardzhishvili, S. Hansen, H. A. Rosenthal, and T. G. Chanishvili, submitted for publication).

A second case of nonclassical M/R is the behavior of T3 towards *E. coli* W1655 (248, 249). Independent of the fact that T3hw host range mutants appear at a frequency of 10^{-8} in the phage population (see Adsorption and DNA Injection), the efficiency of plating of T3 wild-type phage assumes values between 10^{-7} and 10^{-1}, depending on the preceding host. These remarkable differences in efficiency of plating can be attributed quantitatively to different adsorption values (248, 249).

To our knowledge, nonclassical modification of T3 and T7 bacterial viruses constitutes a novel phenomenon in virology. The improvement of adsorption to cells of the same type and the impairment of adsorption to other cell types caused by modification may be related to the long-disputed mechanisms of cell and tissue adaptation of animal viruses which cannot be reduced to mutant selection alone (see Fenner et al. [123] p. 317–318). In addition, nonclassical M/R could interfere with lysotyping of bacteria (247).

REGULATION OF GENE EXPRESSION

Transcription

Transcription of phages T3 and T7 proceeds in distinct phases (Fig. 1): early transcription (class I genes), performed by the host RNA polymerase, succeeded by late transcription (class II and class III genes), carried out by the phage-specified RNA polymerase (484).

Early transcription. Early transcription commences at the left end of the genome, where *E. coli* RNA polymerase recognizes three adjacent promoters, *A*1, *A*2, and *A*3, in vivo (112, 318). The reader is guided to the 1979 review of Rosenberg and Court (406) for thorough information about promoter recognition. All early T7

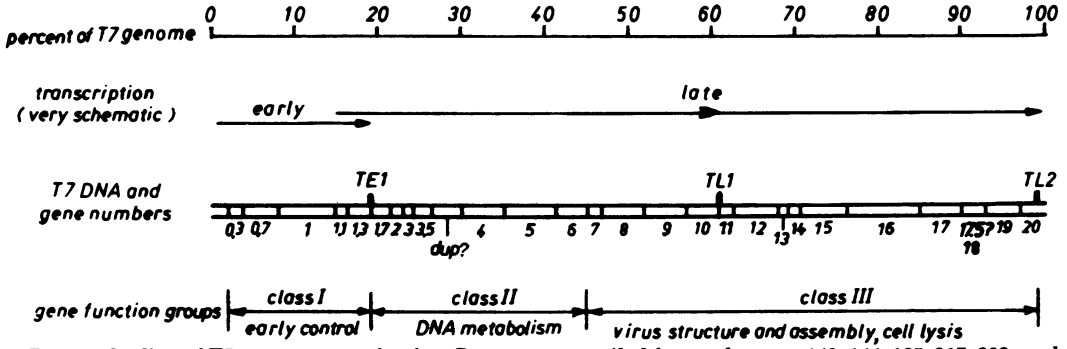

FIG. 1. *Outline of T7 genome organization. Data were compiled from references 143, 144, 165, 217, 302, and 484. For phage-coded proteins, see Table 4.*

promoters (Table 1), with the exception of one minor promoter (*D* or *A0*) set off transcription in left-to-right direction to copy the heavy strand of viral DNA (471, 499). Promoters for bacterial RNA polymerases have in common homologies in functionally important regions which are strongly conserved in evolution (136, 406, 523) (Table 2). The "−35 region" is implicated in initial recognition, and the "Pribnow box" (374, 375, 427) probably triggers local unwinding of the double strand (444). By elegant chemical DNA modification techniques, Siebenlist (457) and Siebenlist and Gilbert (458) identified the minimal unwound promoter *A3* sequence (Table 2) and the contact points of the −35 region and the Pribnow box with *E. coli* RNA polymerase. It has been pointed out that the *C* promoter has the lowest homology to an "average promoter" constructed from superposition of several known promoter sequences, despite the fact that it is relatively strong under certain in vitro conditions (see discussions by McConnell [304] and Pfeffer et al. [362]).

The properties of purified *E. coli* RNA polymerase as well as its interaction with DNA have been reviewed extensively (20, 68, 69, 136, 211, 262, 543). The concentrations and the specificity of bacterial RNA polymerase depend on the physiological state of the cell (2, 73, 130, 219, 274, 329, 392, 506, 507, 543). Since the role of the minor T3 and T7 promoters has not been firmly established, it would be interesting to know whether variant *E. coli* RNA polymerases are capable of recognizing alternative promoters on phage DNA.

The binding of *E. coli* RNA polymerase to T7 DNA has been explored by electron microscopy (36, 74, 87, 88, 232, 370, 525) and with the aid of isolated restriction fragments (148, 200, 202, 276). Improvements of the technique enabled Koller et al. (231) to reliably localize the minor promoters *B* through *E* (Table 1). These authors summarized and unified previously published data on the location of early T7 promoters. Remaining differences, e.g., seven binding sites identified by Koller et al. (231) as opposed to eight found by Hinkle and Chamberlin (190), may be reconciled by the work of Kadesch et al. (212, 213) proving the existence of tight-binding, promoter-like polymerase-DNA complexes distributed nonrandomly over the DNA molecule. The relative "strengths" of class I promoters estimated by three independent methods rise in the sequence $A0 \ll A3 \leq A2 < A1$ (536).

The following steps precede initiation of mRNA synthesis: binding of RNA polymerase holoenzyme at "nonspecific" sites which might serve as storage stretches (426), diffusion to and recognition of promoters, conformational change of the enzyme, and melting of about 10 base pairs (87, 383, 457) of the promoter. At this stage the complex has become relatively stable to rifampin, heparin, polyinosinate, and denatured DNA (20). The rate of attack by rifampin (471) or heparin (362) depends on polymerase conformation, which, in turn, is determined by the structure of the respective promoter. The sensitivity of such initiation complexes in vitro does not correspond to the strength of the promoter (362). The actual initiation reaction is the formation of a dinucleoside tetraphosphate. Dinucleoside monophosphates stimulate rapid RNA synthesis in vitro (87, 289, 318, 339), which,

TABLE 1. *Positions of promoters for E. coli RNA polymerase on T7 DNA*

Promoter[a]	Position (% of genome)[b]	Preceding gene	Copied DNA strand[c]
D(= *A0*)	0.581		L
A1	1.221	0.3	H
A2	1.539	0.3	H
A3	1.834	0.3	H
B	3.728	0.7	H
C	7.821	1	H
E	92.123	19	H

[a] Designation according to Stahl and Chamberlin (471) and Studier et al. (494). These promoters are numbered I through VII by Koller et al. (231).

[b] Data from Koller et al. (231) determined electron microscopically. Errors estimated by these authors are 0.056 to 0.22% of genome length.

[c] L, Light; H, heavy.

TABLE 2. *Sequences of promoters for E. coli RNA polymerase on phage T7 DNA*[a]

Promoter	Sequence (sense strand, 5′ → 3′)
A1	AAAAGAGTA TTGAC TTAAAGTC—TAAC CTA TAG GATACTT ACAGCCA TCGAGAGGGACACGGCG
A2	AAACAGGTA TTGAC AACATGAAG TAAC ATG CAG TAAGATA CAAATCG CTAGGTAACACTAGCAG
A3	ACAAAAC C/G G TTGAC AACATGAAG TAA—ACA CGG TACGATG TACCACA TGAAACGACAGTGAGTC
C	ATAAGCAAC TTGAC GCAATGTTA ATGG GC—TGA TAGTCT— TATCTTA

[a] Promoter sequences were compiled from references 374 (*A3*), 456 (*A1*, *A2*, and *A3*), and 304 (*C*). A or G indicates the first copied nucleotide; the solid box is Pribnow box, and the dotted box encloses the −35 region. Indicated homologies correspond to average promoter sequences (304, 404, 406, 456). Dashes (—) in *A1*, *A3*, and *C* were inserted to achieve exact superposition of homologous regions. The wavy line indicates the minimal unwound sequence (457).

however, does not initiate at the in vivo point (see references in the footnote to Table 2). Using purified *E. coli* RNA polymerase plus adenosine 5'-triphosphate and uridine 5'-triphosphate, Nierman and Chamberlin (330) succeeded in synthesizing T7 mRNA correctly initiated at the *A*1 promoter with pppApU, the natural 5' terminus of *A*1 mRNA (241).

In the elongation complex the DNA region protected by *E. coli* RNA polymerase is shorter (about 26 nucleotides in the coding strand [401]) than in the promoter complex (about 40 nucleotides [374, 375]).

Five early transcripts are made in vivo (39, 40, 112, 113, 177, 462, 486, 494, 497): 0.3, 0.7, 1.0, 1.1, and 1.3 mRNA (Fig. 2). The synthesis of T3 and T7 early messengers terminates either in a rho-factor-independent manner (226) at the major termination site *TE*1, located at 18.9% (376, 494) on the T7 genome, or at terminator sites behind transcriptons 0.3, 0.7, and 1.0 in a rho-dependent process, as shown in vitro (84, 86) as well as in vivo (177). Adhya et al. (6) have identified two rho-dependent termination sites at 8% (after gene 0.7) and 15% (after gene 1) of T7 DNA. For details on mRNA termination, we refer the reader to the review of Adhya and Gottesmann (5). Reinitiation (318) at the beginnings of genes 0.7 and 1.0 (86)—supported by the existence of promoters at these positions (Table 1 and Fig. 2)—could contribute to the pattern of the gene expression.

The major terminator *TE*1 brings most of the transcribing complexes of T7 to a halt in vivo and in vitro (226, 318, 484). The same applies for T3 early termination in vivo (111, 226), but apparently not in vitro (88, 111, 226). *TE*1 recognition in vivo is supposed to be promoted by the phosphorylation of the host polymerase β and β' subunits by the T7 0.7 gene product (gp0.7) (352, 368, 378, 410). Phosphorylation also renders the enzyme unable to reinitiate transcription of host and viral DNAs (546) (see Shutoff of Host and Class I and Class II Phage Functions). Read-through early mRNA terminates at site *TE*2 (30.1%) (318, 360, 462). Sequence analyses of the *TE*1 area of T7 DNA were recently published (116, 376).

Late transcription. Late transcription begins about 4 min (37°C) or 6 min (30°C) postinfection. Besides genes 1.1 to 1.3, the overlap region between class I and class II genes, the latter include genes 1.7 to 10. The first class II gene, immediately following *TE*1, has only been identified by DNA sequence analysis and precedes gene 1.7 (34, 116). Genes 7 and 10 are transcribed from both class II and class III promoters (303) (Fig. 1). By 1979 the primary structures of 10 late T7 promoters were elucidated (Table 3). These structures are shorter and mutually more homologous than promoters for bacterial polymerases (see Table 2). Regions of hyphenated twofold symmetry discovered in the 70% promoter by Oakley and Coleman (336) occur in all sequenced promoters and may contribute to promoter strength, since they are less pronounced in the class II promoters (Table 3). Class II promoters appear weak in vitro (22, 143, 144, 332, 463) but not in vivo (302, 346). Effective and specific transcription from class II promoters in vitro can only be accomplished by using restriction fragments lacking class III pro-

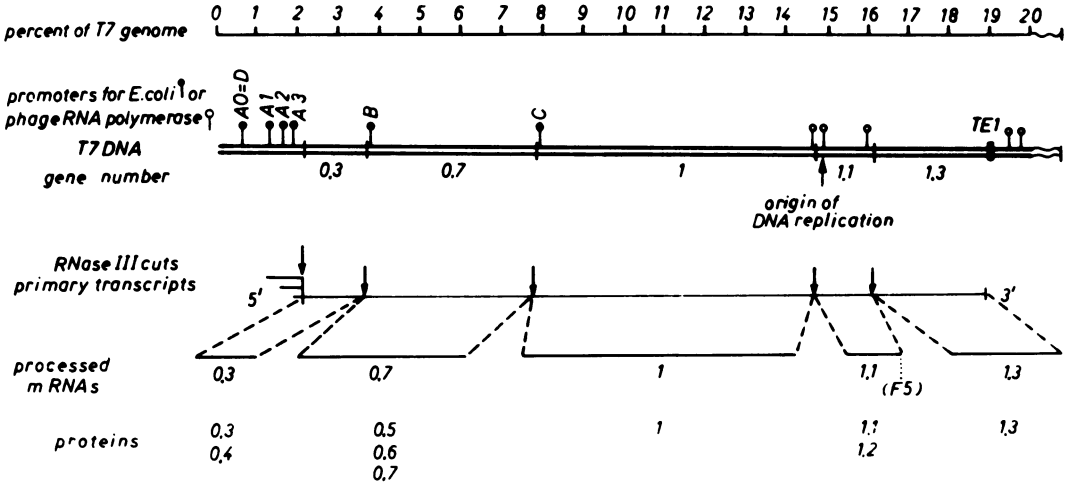

FIG. 2. *Early region of phage T7 and its expression. Data compiled from references 34, 108, 112, 116, 217, 231, 276, 336, 354, 356, 398, 407, 418, 462, 491, 493, 494, and 503.*

TABLE 3. *Positions and sequences of promoters for T7 RNA polymerase on T7 DNA*

Class	Position (% of genome)	mRNA species in vitro[a]	Sequence (sense strand, 5′ → 3′)[b]	Reference(s)
II	14.55	VII	CAATACGACTCACTATAGAGGGA	338, 355, 404, 494
II	14.75	VII	TAATACGACTCACTATAGGAGAA	355, 418
II	15.95	VII (?)	TAATACGACTCAGTATAGGGACA	116, 355
II	19.36	VIII	TAATACGACTCACTAAAGGAGGT	34, 116
II	19.65	VIII	TAATACGACTCACTAAAGGAGAC	34, 116
II	33.3	II_b (?) (in vivo)	AATACGACTCACTAAAGGAXXC	Carter et al.[c]
II	34.75	II_b (?) (in vivo)	TATTCGACTCACTATAGGAGAT	Carter et al.[c]
III	46.5	III_a	TAATACGACTCACTATAGGGAGA	404
III	55	IV	TAATACGACTCACTATAGGGAGA	404
III	57	V	TAATACGACTCACTATAGGGAGA	404
III	70	II (?)	ACATACGACTCACTATAGGGAGA	338
III	87	III_b	TAATACGACTCACTATAGGGAGA	404

[a] mRNA's are designated according to McAllister and McCarron (302), Kassavetis and Chamberlin (217), and Pachl and Young (346).

[b] The wavy line indicates the unwound sequence (481). The position of the initiation nucleotide (A) is questionable (see references 32, 34, 355, 404, 481).

[c] A. D. Carter et al., Abstr. Miami Winter Symp., 12:103.

moters (217), indicating that a special mechanism exists in vivo to activate class II promoters.

Since the first class II promoters are located immediately adjacent to gene 1, coding for phage RNA polymerase (418) it is probable that the enzyme is still physically connected to the gene (via mRNA and the ribosome) when it initially binds to the promoter. This mechanism of self-recognition is a general regulatory principle operative in procaryotes to ensure specificity (444).

Rosa (404) calculates that no more than 24 nucleotides in advance of the initiation site are necessary for promoter function. In her opinion the adenine- and thymine-rich sequence between −13 and −20 might be analogous to the −35 region, and the sequence TATAGGGA might be analogous to the Pribnow box TATPuATPu of promoters for bacterial RNA polymerase.

T3 and T7 RNA polymerases are monomeric enzymes of 97,000 and 105,000 daltons, respectively (Table 4). Both enzymes are specific for homologous DNA, that of T3 more strictly so than the T7 enzyme (22, 24, 66, 109, 145, 170, 281-283, 422, 430; Beier, Ph.D. thesis). They recognize neither host polymerase-specific promoters nor terminators (143, 144, 346) and are not amenable to rho-factor action (66).

Like practically all nucleotidyltransferases, T7 RNA polymerase is a zinc metalloenzyme (77), and like most other early T7 enzymes, RNA polymerase is found associated with the cell membrane (120). The initiation of RNA synthesis by the phage enzyme (301) is basically similar to the mechanism described for *E. coli* RNA polymerase (330); however, the polymerase-DNA complexes are unstable until a short oligonucleotide has been synthesized (65, 301). Like the bacterial RNA polymerase, the T7 enzyme binds to the coding strand and melts a 10-base-pair sequence of the promoter (479) (see Table 3). RNA synthesis catalyzed by the T3 and T7 enzymes is 5–10 times faster than that of the bacterial polymerase (283, 301).

The specificity of promoter recognition by T3 and T7 RNA polymerase has been studied from different angles. Stahl and Chamberlin (472) showed that modification of the minor DNA groove by base analogs prevents utilization of promoters by T7 RNA polymerase, but alterations in one strand affecting the major groove do not. The properties of hybrid RNA polymerases induced by recombinants of T3 and T7 DNA within gene 1 were analyzed by Hausmann, Beier, and co-workers (22, 24, 170). The lower molecular weight of the T3 enzyme results from one or two small deletions in the T3 gene 1 when aligned with T7 gene 1 (24). Template preference seems to be imparted by the region from 0.70 to 0.78 gene 1 length units. The relative activity on heterologous phage DNA was found to vary from 20 to 80% among different recombinant RNA polymerases, in comparison with 15% for T3 and 50% for T7 wild-type RNA polymerases, and could not be ascribed to a particular region of the enzyme (170). In any case gene 1 recombinants are only viable if the template specificity domain of the RNA polymerase matches a minimum of promoter sequences within the recombinant DNA (22).

Chain initiation by T3 or T7 RNA polymerase is highly specific: the 5′ terminus of all in vitro-synthesized mRNA is $pppGp(Gp)_nA$ in the case of the T3 (283) or $pppGpGp(Ap)_n$ in the case of the T7 (32) enzyme. Termination of RNA synthesis, at least by T3 RNA polymerase, appears

TABLE 4. *T7- and T3-directed proteins*[a]

Protein (gp) no.	Name or function of protein	Mol wt of protein (reference)		Constituent of the mature virion	Reference(s)
		T7	T3		
0.3	*ocr* protein (*ocr* and SAMase for T3)	8,700	11,500	No	254, 487, 493
0.4	Unknown	6,600[b]	ND[c]	No	110, 474, 494
0.5	Unknown	ND	ND	No	494
0.6	Unknown	ND	ND	No	494
0.7	Protein kinase	42,000	40,000	No	44, 369, 352, 378, 485, 486, 410, 493
1	RNA polymerase	105,000[d]	97,000	No	24, 70, 109, 333, 493; Issinger[e]
1.1	Basic protein (DNA initiation?)	5,700[b]	ND	No	418, 494
1.2	DNA initiation?	12,000[b]	ND	No	418, 494
1.3	DNA ligase	40,000	37,000	No	293, 346, 484, 485, 493
1.7	Unknown	17,000	ND	No	484
2	Inhibitor protein	8,500	ND	No	96, 185, 267
3	Endonuclease I	13,500	ND	No	61, 484
3.5	DNA and RNA metabolism (formerly called "lysozyme")	17,000	ND	No	228, 303, 346, 460, 484
dup?	DNA-unwinding (binding) protein	31,000	ND	No	165, 345, 385, 438
4	Primase	66,000 58,000[f]	ND	No	188, 233, 434, 192, 484
5	DNA polymerase subunit, 3′-5′ exonuclease	87,000	ND	No	197, 346, 388, 484
6	Exonuclease	31,000	ND	No	346, 484, 539
7	Coat protein (infectivity)	14,700 (484) 50,000 (207)	55,000	Yes	207, 319, 400, 484
8	Head protein	67,000	85,000	Yes	207, 319, 346, 400, 484
9	Head assembly (scaffolding) protein	45,000[d]	ND	No	207, 319, 346, 400, 484
10	Major head protein Minor head protein	38,000[f] 45,000	50,000	Yes	207, 319, 346, 400, 484
11	Tail protein	22,000[d]	27,000	Yes	207, 319, 400, 484
12	Tail protein	92,000[d]	100,000	Yes	207, 319, 400, 484
13	Coat protein (infectivity)	14,000	13,000	Yes	207, 319, 400, 452, 484
14	Head protein (core)	20,000[d]	20,000	Yes	207, 319, 400, 452, 484
15	Head protein (core)	89,000[d]	96,000	Yes	207, 319, 346, 400, 452, 484
16	Head protein (core)	170,000[d]	180,000	Yes	207, 319, 346, 400, 452, 484
17	Tail protein (fibers), "serum blocking power protein"	65,000 (346) 76,000 (207, 484)	80,000	Yes	206, 207, 319, 346, 400, 484
17.5 ?	Lysis enzyme	ND	ND	?	320, 325
18	DNA maturation and packaging	ND	ND	No	319, 400, 484
19	DNA maturation and packaging	73,000	ND	No	319, 346, 400, 484
20	DNA packaging in lambda-lysogenic cells	ND	?	?	358

[a] Molecular weights are either the latest published data, averages of published data, or, when differences between published data were too great, both currently accepted values. Recently identified small phage proteins of unknown function (34, 494) are not included.

[b] Computed from published DNA sequence (238, 418).

[c] ND, Not determined.

[d] Average data.

[e] O. G. Issinger, Ph.D. thesis, University of Freiburg, Freiburg, Federal Republic of Germany.

[f] Possibly processed.

to occur with comparable precision independent of other protein factors, yielding the 3′ sequence $Gp(Up)_5G_{OH}$ (285).

T3 and T7 RNA polymerases are salt sensitive in vitro (20, 65, 66, 109, 281, 430). The salt sensitivity of late T3 transcription in vitro can be overcome by a specific interaction of *E. coli dnaB* protein with T3 DNA (S. Michel and B. Brux, Abstr. Commun. Annu. Meet. Biochem. Soc. GDR, 11th, Halle/Saale, German Democratic Republic, 1979, E14; S. Michel, personal communication). By the following criteria this interaction appears also essential to relieving the salt sensitivity of late transcription in vivo: *E. coli* CRT 266, a *dnaB* mutant producing a thermosensitive *dnaB* protein, is nonpermissive for T3 replication at high temperature (290). Under nonpermissive conditions, only the early T3 proteins are expressed, and temperature shift experiments indicate that the *dnaB* protein is required for the initiation of RNA chains by T3 RNA polymerase. RNA synthesis in toluenized T3-infected *dnaB* mutant cells is stimulated by salt at permissive temperatures, but inhibited by salt at restrictive temperatures. Added wild-type *dnaB* protein reverses salt inhibition of purified T3 RNA polymerase at 42°C, whereas the thermosensitive *dnaB* mutant protein fails to do so (Michel and Brux, Abstr. Commun. Annu. Meet. Biochem. Soc. GDR, 11th, Halle/Saale, 1979, E14). One could surmise an analogy between the interaction of T3 RNA polymerase with *E. coli dnaB* protein, on the one hand, and that of *E. coli* primase with *dnaB* protein, wherein they form a "mobile replication promoter" (10), on the other.

Class III transcription encompasses all genes to the right of gene 6 (see Fig. 1). In vitro T3 RNA polymerase synthesizes six (145), seven (22, 285), or eight major transcripts (64, 65, 284) which are translatable by in vitro translation systems to phage proteins (65). T7 RNA polymerase generates six (333) or seven (143, 302) major mRNA species in vitro which are all class III (22, 332, 345). Besides that, three minor transcripts, A, B, and C, are detected which extend from promoters between 45 and 60% to the 99% termination site (302). Similar large-size transcripts (I, II, and III) are also generated by T3 RNA polymerase in vitro (284).

Late transcription terminates at only two specific sites (*TL*1 and *TL*2) in vivo and in vitro: approximately 55 and 100% in the case of T3 (22) and 61 plus 99% in the case of T7 (302) (see Fig. 1).

Pachl and Young (346) have recently determined the sizes and mRNA activities of T7 late in vivo transcripts (eight from class II and eight from class III) in an in vitro translation system and compared their data with the previously reported (22, 143, 144, 332) activity of mRNA synthesized in vitro. T3 and T7 in vivo transcripts either are identical to the in vitro transcripts or seem to be derived from them by ribonuclease (RNase) III digestion (see Messenger Ribonucleic Acid Processing and Translation). Among the in vivo transcripts it is possible to distinguish several class II mRNA's (155, 345, 346); these mRNA's cannot be fractionated from T7 mRNA synthesized in vitro (143, 144, 332) (see above). In T7-infected RNase III-negative host cells, Hagen and Young (155, 156) have detected T7 messengers as large as 4×10^6 to 5×10^6 daltons coding for gene 3.5 (class II) protein, indicating that class II mRNA synthesis in vivo terminates behind gene 10, at the first (*TL*1) of the two unique termination sites (Fig. 1).

Messenger Ribonucleic Acid Processing and Translation

The early region of the T3 or T7 genome is transcribed into a large polycistronic messenger (39, 40, 112, 113, 177, 462, 497) which in vivo is processed by the host RNase III to yield 2 mono- and 3 polycistronic mRNA molecules (Fig. 2). The work on RNase III action not only is fundamental for the comprehension of the molecular biology of T3 and T7 but also has pioneered the study of sequence-specific RNA cleavage in eucaryote systems (1, 397). Thus, several publications were dedicated to the mechanism of site-specific RNA processing of T3 and T7 RNAs by RNase III (107, 108, 114, 178, 240, 241, 398, 408, 409, 491). The recognition sequences of RNase III were identified which presumably adopt a hairpin conformation (110, 114, 241, 336, 398, 407–409, 491). In vitro it is possible to generate cuts at secondary sites by lowering the ionic strength, whereby, for instance, fragment F5 is cleaved off the 1.1 mRNA (Fig. 2). Secondary processing is, however, relatively ineffective in vivo (107, 398). It is surprising that, in a situation resembling a primitive version of that in eucaryotic cells, the 3′ ends of processed mRNA are subsequently oligoadenylated (241, 407). T3 RNA polymerase catalyzes polyadenylate synthesis in vitro (422), but its involvement in oligoadenylation of phage mRNA has not been proven.

Hercules et al. (178) postulated that processing of the polycistronic early T3 and T7 messengers was necessary for their effective translation. Other authors (108, 114, 115, 488, 533) do not generally confirm this opinion, but find that the efficiency of 0.3 protein synthesis is increased by

RNase III cleavage of polycistronic mRNA, and possibly RNase III processing represents an evolutionary advantage under suboptimal growth conditions (9, 115, 178). The normally about fourfold excess of 0.3 mRNA over the other four early mRNA's is due not only to RNA processing but also to the rho-factor-mediated termination mentioned above.

Steitz and Bryan (474) and Dunn et al. (110) have studied mRNA binding and initiation of protein synthesis on T7 0.3 mRNA. Phage T7 0.3 mRNA is approximately 600 nucleotides long and carries the information for two proteins: the 5′ two-thirds of the messenger specify the gp0.3 (see Interactions with the DNA Modification and Restriction System of the Host: Functions of Gene 0.3), and the remaining part codes for a protein of unknown function referred to as gp0.4. gp0.3 is made in large amounts during T7 infection, gp0.4 is made in smaller amounts, and each of these proteins is synthesized from separate specific ribosome-binding and initiation sites on the 0.3 mRNA. The proximity of the ribosome-binding site for gp0.3 synthesis to the RNase III cleavage site on the left end of gene 0.3 (474) may explain why RNase III action removing a nontranslated 5′ RNA sequence is required for efficient 0.3 translation (115, 491).

Recently Studier et al. (494) demonstrated that not only 0.3 mRNA but also the 0.7 and 1.1 mRNA's encode more than one protein (0.7 mRNA encoding gp0.5, gp0.6, and gp0.7 and 1.1 mRNA encoding gp1.1 and gp1.2). Thus, the early region of the T7 genome codes for nine proteins. The functions of gp0.3 (*ocr* protein), gp0.7 (protein kinase), gp1.0 (RNA polymerase), and gp1.3 (DNA ligase [polydeoxyribonucleotide synthetase]) are ascertained, whereas nothing is known about the functions of the 50- to 120-amino-acid proteins 0.4, 0.5, 0.6, 1.1, and 1.2. For eight of these proteins (but not yet for gp1.2) the existence of special ribosome-binding and initiation sites which are apparently used with different efficiencies has been established (110, 747, 494). There is no evidence of any proteins being specified by the region between the *A* promoters at the far left of the genome (see Table 1 and Fig. 2) and the beginning of gene 0.3 (494).

Not only the early transcripts but also polycistronic class II and class III late T3 and T7 mRNA's are subject to RNase III processing (114, 157, 284, 346).

Shutoff of Host and Class I and Class II Phage Functions

Gene expression of T3 or T7 phage is regulated mainly on the level of transcription (177, 196, 478, 489). The early (class I) gene products serve to create an environment favorable for phage-specified syntheses. Then the class II proteins responsible for the synthesis and maturation of DNA appear. As soon as sufficient amounts of these proteins are present, class I and class II transcriptions are turned off to ensure that the bulk of amino acids provided by the host cell are converted into phage structural and maturation proteins (class III proteins) (see Fig. 1 and Table 4).

The times of appearance and the concentrations of phage proteins are in essencé determined by the positions of the respective genes in relation to promoters and termination sites. This does not apply only to their expression in one of the three classes: the dosages of certain proteins required for a longer period (possibly in the 1.1 to 1.3 region?) or in a very high number (the major head protein coded by gene 10) are enhanced because their genes are localized in areas of overlapping transcription—they are transcribed from early and late (genes 1.1 and 1.3) or from class II and class III late promoters (genes 7 to 10). Concentration differences between mRNA's and proteins of one class are also maintained by overlapping transcription from alternative promoters of this class. Thus, due to intermittent termination, promoter-proximal genes are transcribed more often than promoter-distal genes. The economy of phage syntheses displays such a degree of perfection that apparently genes 18 and 19, coding for maturation proteins (see Phage Morphogenesis and Cell Lysis), are transcribed only for the short period between the initiation of class III transcription and the termination of class II transcription (472, 484).

The most important regulatory principle consists in the induction of a phage-coded RNA polymerase with a strict specificity for homologous DNA and concomitant inactivation of the host RNA polymerase. Two phage proteins are involved in the inactivation of *E. coli* polymerase, first of all the T7 seryl-threonyl protein kinase (adenosine-5′-triphosphate-protein phosphotransferase) specified by gene 0.7 (352, 369, 378), which phosphorylates the β and β' subunits of *E. coli* RNA polymerase (546). The reason why the phosphorylated host enzyme becomes less active is not quite clear and cannot be explained satisfactorily by the recognition of the terminator *TE*1 by the modified polymerase (369) and its inability to reinitiate RNA synthesis (546). Recent studies by Hesselbach and Nakada (184) and McAllister and Barrett (298) confirm the role of the T7 protein kinase in the shutoff of *E. coli* RNA polymerase-dependent transcription. It is probable that the early T3 function capable of altering the β subunit of *E. coli* RNA polymerase and reducing polymerase

activity to 25% (100) is analogous to the T7 gene 0.7 function (despite the fact that these authors could not detect a corresponding activity in T7-infected cells).

gp0.7 phosphorylates not only the host's RNA polymerase but also other proteins in the infected cell (377), including itself in a self-inactivating reaction (352). Rahmsdorf et al. state that their discovery of T7 kinase is the first demonstration of a seryl protein kinase—a well-known enzyme activity in eucaryotes—in a procaryotic cell (378).

A much stronger effect on *E. coli* RNA polymerase is, however, exerted by an inhibitor protein coded for by gene 2 (184). This inhibitor protein (see Table 4) binds physically to RNA polymerase holoenzyme, forming a 1:1 complex (96, 185, 267). The sensitivity of the wild-type RNA polymerase is apparently dependent on the σ subunit, but its sensitivity can be abolished by *tsnB* and BR3 mutations affecting the β subunit of the enzyme (96) (see *tsn* Mutations of *Escherichia coli*). It is not clear whether a connection exists to the loss of the σ factor by *E. coli* RNA polymerase holoenzyme after T7 infection reported by Khourgess and co-workers (33, 548). gp2 has no effect on phage RNA polymerase (183–186, 267, 368, 369). An analogous late protein which blocks the initiation of RNA synthesis by *E. coli* RNA polymerase was also described for T3 (279, 280) and is probably T3 gp2.

In contrast to the 0.7 protein kinase, gp2 is essential for phage development (484). The exceeding complexity of gp2 functions (this protein is also required during DNA maturation [see DNA Replication, Maturation, and Recombination]) have made it difficult to pinpoint the essential interaction(s). It is clear, though, that binding of gp2 to *E. coli* RNA polymerase is essential, since the above-mentioned *tsnB* mutants (67) do not support the growth of T7 phage (96).

The shutoff of host and early phage protein syntheses is not entirely explained by the mechanisms described above. Two further processes may be involved in the early-to-late switch: translational discrimination in favor of late mRNA and functional instability of early mRNA's. Some authors (441, 536–538) have estimated the functional half-life of early mRNA to be only a few minutes, and, therefore, when early transcription is shut down, the cessation of early protein synthesis is inevitable. Results implicating an additional phage-directed control mechanism (protein) preventing the translation of early phage mRNA (179, 446) have been contradicted (532). The situation is complicated by the fact that Young and co-workers report longer mRNA half-lives (196, 346, 478, 479) than those reported by Yamada's group (536–538) and postulate a translational control at least for the expression of gene 0.3 (478). Results of Strome and Young (480) do indicate a translational discrimination against 0.3 mRNA towards the end of the infectious cycle, which, however, is not caused by a phage-specific control protein but simply by the excess of late mRNA. Hercules et al. (177) confirmed the rapid functional inactivation found by Yamada et al. (536–538) and could not trace any translational discrimination among the individual early mRNA species. However, significant differences in translational utilization were reported for early mRNA's by Steitz and Bryan (474), Dunn et al. (110), and Studier et al. (494) and for late mRNA's by Niles and Condit (332). The patterns of in vitro protein synthesis, using late T7 mRNA's, show a striking correspondence to in vivo patterns (196, 332, 345, 346); hence, additional modes of "late" translational control in the infected cell probably do not exist.

The predominance of class III protein synthesis over class II protein synthesis (484) might in part result from a faster functional inactivation of class II mRNA's, though data on this topic are scarce (154). McAllister and Wu (303) have now demonstrated that not only the synthesis of late proteins but also late transcription itself is temporally regulated: class II and class III mRNA's are synthesized at different times and in different amounts. This regulation is partially mediated by gp3.5, since in its absence the turnoff of class II transcription at 15 min postinfection (30°C) is not performed (303).

gp2 is also suspected to play a role in this switchover. It has been proposed that complexes of gp2 with *E. coli* RNA polymerase might bind to sites in the late genome region and inhibit progression of the phage-coded RNA polymerase (218, 471). This mechanism is reminiscent of the action of repressor-like polymerase-rifampin complexes on T7 mRNA synthesis in vitro (35, 218), and, in fact, one of the activities of gp2, namely, in DNA packaging in vitro (see below), can be replaced by rifampin (267, 344). In line with this evidence, the class II-type shutoff of gene 19 transcription (472, 484) might be accomplished by an inhibitory *E. coli* polymerase complex bound to promoter *E* (see Table 1 and Fig. 1).

In summary, the following mechanisms may, in principle, contribute to the observed concentration differences between individual phage proteins in the infected cell: (i) promoter utilization and termination (transcription rate), (ii) mRNA half-lives, (iii) translational utilization of individual messengers, and (iv) functional stability of the proteins themselves.

INTERACTIONS WITH THE DNA MODIFICATION AND RESTRICTION SYSTEM OF THE HOST: FUNCTIONS OF GENE 0.3

Adenosylmethionine Hydrolase

T3 encodes an enzyme, SAMase (EC 3.3.1.2), which hydrolyzes intracellular *S*-adenosylmethionine (SAM), yielding thiomethyladenosine and homoserine (131, 141, 142). The corresponding gene (*sam*$^+$) is located at the left end of the genome (168) and was numbered gene 0.3 (493, 496). The molecular weight of the T3 protein is 11,500 (493), but a higher molecular weight, 17,000, has also been reported (469). T7 does not induce a SAMase, although a gene 0.3 is present (131, 141, 142, 254, 462, 470, 484, 487, 494).

SAM is the methyl donor for the transmethylation of macromolecules (DNA, RNA, protein); it is involved in the synthesis of polyamines and in other biosynthetic pathways (52, 511). SAM plays a key role in the processes of host-controlled DNA M/R; classical modification is based on DNA methylation, and, moreover, type I restriction enzymes require SAM as an allosteric effector for DNA binding or the stabilization of this interaction (11, 153, 504, 542).

Although Hausmann (161) isolated a number of T3*sam*$^-$ mutants, the biological functions of SAMase remained obscure for a long time, since *sam*$^+$ and *sam*$^-$ T3 derivatives behaved identically on a variety of host cells and under different growth conditions (164). In 1975 the biological consequences of SAMase action were recognized by Krüger et al. (243). In starved cells T3 wild-type phage becomes temperate, whereas T3*sam*$^-$ and T7 multiply normally. When, however, methylation of T3*sam*$^-$ phage DNA is prevented, the mutant phage also establishes a lysogenic infection (see T3 As a Facultative Temperate Phage). A second trait conferred by the possession of SAMase is the partial protection of T3 against in vivo restriction by *Eco*P1 endonuclease (243, 251), probably because the lack of SAM in the T3-infected cell prevents stable binding of the restriction endonuclease to phage DNA (see P1 Prophage).

The possibility of turning off all SAM-dependent processes with the aid of the *sam*$^+$ gene makes phage T3 a convenient tool to study the consequences of preventing DNA methylation, type I restriction, and other cellular SAM-dependent reactions (82, 141, 149, 227, 243, 288, 425). Doubtless, this is an interesting technique, but it must be taken into account that, for example, DNA M/R is counteracted not only by SAMase but also by a second function localized in gene 0.3, the *ocr*$^+$ function (see Ability to Overcome Classical Restriction (*ocr*)).

For a long period T3 was believed to be the only phage to encode SAMase (164, 203). Meanwhile, it has been demonstrated that coliphage S_D, *Serratia* phage IV, and *Klebsiella* phage 11 produce SAMase (239, 331).

Ability to Overcome Classical Restriction (*ocr*)

T3 and T7 can be passaged through *E. coli* strains of different DNA host specificities (e.g., *E. coli* strains C, B, and K) without being phenotypically restricted (122). This property is not unique to T3 but is also displayed by T7 and T3*sam*$^-$ derivatives, which means that even in the absence of SAMase there is sufficient protection against DNA restriction, despite the fact that both T3 and T7 DNAs contain recognition sites for *Eco*B (121, 254).

Studier (487) and Studier and Movva (493) showed that T3 and T7 gene 0.3 mutants are phenotypically restricted by *E. coli* strains B and K and carry the respective modifications after passage over these strains. After this basic discovery, the protection mechanism was studied intensively. T3 specifies the SAMase (*sam*$^+$ gene function) as well as the ability to overcome classical restriction, which was termed *ocr*$^+$ by Krüger et al. (254). It was demonstrated (254) that *sam*$^+$ and *ocr*$^+$ are distinct functions of the T3 gene 0.3; T7 only possesses the *ocr*$^+$ function. The *ocr* mutants of T3 and T7 are subject to classical M/R via DNA methylation versus endonucleolytic cleavage (254). The effect of *ocr*$^+$ consists in actively turning off the cellular restriction enzyme to protect the unmodified recognition sites on the phage DNA against cleavage. This protection is, moreover, extended to any foreign DNA simultaneously introduced into the cell. When T3 or T7 phage are inactivated by ultraviolet radiation so that they can no longer destroy the cell but still are able to express gene 0.3, then it is possible to introduce plasmid DNA by conjugation (244) or transformation (387) without it being restricted. The recipient cells survive and are able to replicate themselves as well as the newly acquired plasmid (Fig. 3).

T3 and T7 are the first phages whose mechanisms of turning off cellular restriction enzymes were closely examined (see Modes of Action of *sam*$^+$ and *ocr*$^+$ Gene Functions). Protection is definitely exerted against type I restriction, and an effect against type II restriction (*Eco*RII) is indicated by preliminary results (Wackernagel, personal communication). Meanwhile, antire-

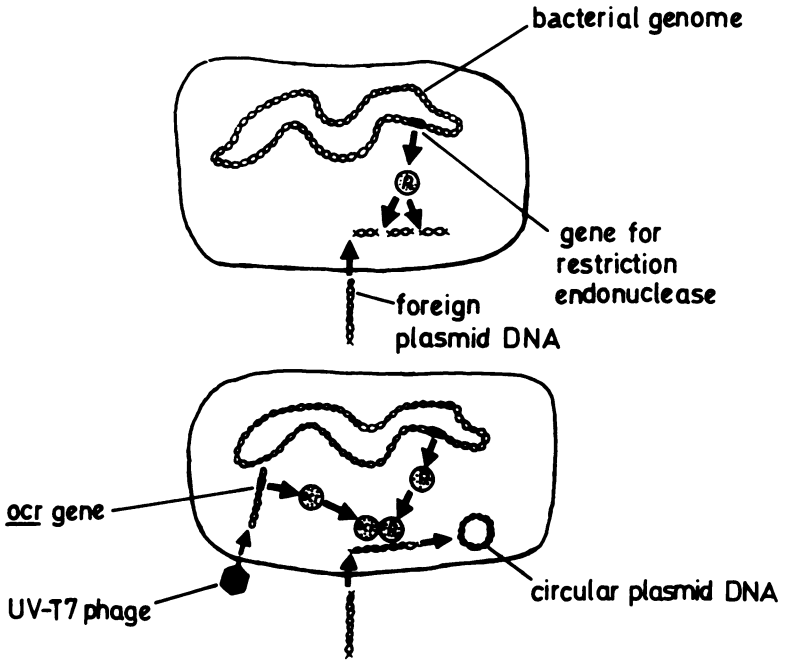

FIG. 3. *Protection by T3 or T7 ocr$^+$ gene function of foreign plasmid DNA against restriction. Plasmid DNA, a common vector in gene transfer, is restricted in normal restriction-proficient recipient cells if it contains recognition sites for the appropriate restriction system (top). After preinfection of cells with ultraviolet (UV)-inactivated T3 or T7 phage still able to express the ocr$^+$ gene, the cellular restriction endonuclease (R) is blocked; subsequently introduced foreign plasmid DNA is not destroyed (bottom). (From references 244 and 387.)*

striction functions of other phages have been described, e.g., for T5 (42, 90, 91) and *Bacillus subtilis* phage φNR2 (287). The situation is much more complex in the case of T-even phages, where in addition to the glucosylation of hydroxymethylcytosine, further protective functions exist (99, 270).

Recently, a function of restriction endonuclease *Eco*K in the in vivo repair of DNA cross-links in *E. coli* was reported (25) where the restriction enzyme acts not nucleolytically, but rather as a regulatory protein or by protecting single-stranded DNA against nuclease attack (26). The ocr$^+$ gene function does not diminish this effect of *Eco*K (A. A. Belogurov, G. B. Zavilgelsky, and D. H. Krüger, submitted for publication).

Effects on DNA Modification

The expression of SAMase during T3 infection causing a depletion of SAM explains why T3 DNA carries no methyl groups whatsoever (131, 141). In contrast, the DNA of the SAMase-negative phage T7 is methylated (131, 141). However, T7 DNA, too, is undermethylated compared with host cell DNA: the ratios of 6-methylaminopurine to adenine and of 5-methylcytosine to cytosine in *E. coli* DNA are about 30 to 50 times higher than those in T7 DNA (U. Günthert, Ph.D. thesis, University of Tübingen, Tübingen, Federal Republic of Germany, 1975). Two reasons for this have been recognized. First, the gene 0.3 ocr$^+$ function prevents not only host-controlled restriction but also modification (245). This is why T7 lacks host-specific (*hsp*) methylation, which is present in the DNA of ocr$^-$ mutants (245, 254, 487). The prevention of *hsp* methylation by the ocr$^+$ function is the explanation of the observation (121) that the DNA of T7 wild-type virus grown in *E. coli* B is susceptible in vitro to cleavage by purified restriction endonuclease *Eco*B.

Second, it was shown (102) that the *dam* methylase (deoxyadenosine methylase [291]) acts only weakly on T7 DNA in vivo: the recognition sequence 5'-GATC-3' for *Mbo*I (135), which happens to be identical with the sequence methylated by the *dam* enzyme (133, 160), is not protected against *Mbo*I cleavage in vitro. This result is supported by data proving that the *Dpn*I endonuclease, which cuts at methylated 5'-GATC-3' sites, scarcely attacks T7 DNA in

vitro, whereas the complementary enzyme *Dpn*II, which specifically cleaves the nonmethylated identical sequence, accepts T7 DNA as a substrate (257, 258). Considering that *E. coli dam* enzyme sensitizes DNA to the action of *Dpn*I (515), one can deduce that during T7 DNA replication *dam* acts on very few, if any, of the *dam*-specific sites in T7 DNA. It is speculated that T7 may replicate faster than its DNA can be methylated by this enzyme (258), or, more probable, that the *dam* protein is an integral component of the *E. coli* DNA replication complex, but not of the T7 replication apparatus, and therefore has no access to phage DNA (102).

Unfortunately, the situation has been somewhat confused by a recently published paper of Auer et al. (15) in which it is stated that the gene 0.3 product (termed "M" protein by this group [see below]) has no influence on the methylation of T7 DNA. The mutants used in this study were merely selected for their inability to grow on a restriction-proficient *E. coli* host and are not phenotypically modified in vivo (15). Consequently, no difference in the DNA methylation of such mutants as compared with the wild type is to be expected. Of course, authentic gene 0.3 *ocr*$^-$ mutants undergo host-specific modification (245, 254, 487, 493). A comparison of the mutants used by this group (15, 193, 364, 366, 446) with the precisely mapped and well-characterized gene 0.3 mutants of Studier and co-workers (487, 493, 494) is called for.

Modes of Action of *sam*$^+$ and *ocr*$^+$ Gene Functions

In T3, *sam*$^+$ and *ocr*$^+$ represent distinctive functions of the 0.3 protein. T7 gp0.3 has only the *ocr*$^+$, not the *sam*$^+$, function. The two functions interfere with different steps in the interaction between DNA and type I restriction enzymes. By destruction of intracellular SAM, the *sam*$^+$ function ab initio suppresses DNA methylation. The activation of type I restriction enzymes and the stability of the enzyme-DNA complex, which both depend on SAM, are reduced in the SAM-depleted cell. The effect of the *ocr*$^+$ function is not connected with SAM hydrolysis and interferes with a later step of the interaction between restriction endonuclease and DNA, preventing cleavage as well as methylation (245, 251, 254; D. H. Krüger, Int. UNESCO/ICRO Symp., Szeged, Hungary, 1977). The independence of the *sam*$^+$ and *ocr*$^+$ activities has recently been confirmed by in vitro studies on purified gp0.3's from T3- and T7-infected cells (470). Thus, T3 gp0.3 appears to be at least bifunctional. The isolated 0.3 proteins of T3 and T7 bind directly to the restriction endonucleases *Eco*B and *Eco*K and exert *ocr* activity in this way (470; F. W. Studier, personal communication).

Cellular restriction enzymes usually destroy foreign DNA immediately upon its entry into the cell (461). The *ocr*$^+$ function bestows complete resistance to endonucleolytic cleavage to the phage DNA (254), and this protection depends on active synthesis of gp0.3 in the infected cell (423; D. H. Krüger, unpublished data). We have already mentioned that gene 0.3 is located on the far left end of the genome, which is the first to be injected (357, 417) and expressed (168, 462, 487, 493, 497). Furthermore, T7 (and maybe also T3) 0.3 mRNA has an increased rate of synthesis and is more stable than the other early mRNA's (see Regulation of Gene Expression), which leads to an especially high rate of gp0.3 synthesis (177). But how can the *ocr*$^+$ function ensure instantaneous protection of the phage DNA when gp0.3 only becomes detectable at 2 min (37°C) in postinfection (168, 177)?

The delayed injection of T3 and T7 DNAs may be the "trick" by which the phages prevent exposure of DNA recognition sites before the appearance of gp0.3. Transcription of the leftmost DNA region must be the precondition for injection of the rest of the genome. This has been elegantly confirmed by experiments showing that upon blocking of *E. coli* RNA polymerase by rifampcin or streptolydigin, injection of T7 DNA is inhibited (544). The development of the antirestriction function of phage T5 follows a similar schedule (42, 90, 91).

The two gene functions *sam*$^+$ and *ocr*$^+$ make T3 and T7 interesting model viruses for resolving individual steps of DNA M/R and their modulation by virus functions. They could also be useful in elucidating the in vivo significance of sequence-specific DNA methylation and cutting processes for DNA replication, repair, recombination, and transcriptional control (12, 25, 48, 81, 92, 137, 194, 261, 382, 396, 512; T. A. Trautner, B. Pawlek, U. Günthert, U. Canosi, S. Jentsch, and M. Freund, Mol. Gen. Genet., in press; see also T3 as a Facultative Temperate Phage). It was discovered recently that SAM-dependent endonucleases corresponding to certain methylases also exist in eucaryotic cells (47).

Other Functions Ascribed to Gene 0.3

Ratner (380) found that T7, but not T3, gp0.3 binds to *E. coli* RNA polymerase. The nature of this interaction has not been studied. On the basis of this observation it was supposed for a certain time (183) that the inhibitor protein later identified as gene 2 product (184) was encoded by gene 0.3.

In 1974 Herrlich et al. (179) claimed to have localized the T7 gene coding for a translational repressor (see Shutoff of Host and Class I and Class II Phage Functions) on the left end of the T7 genome, between the early promoters and the 0.7 (kinase) gene. However, in the year thereafter Herrlich and co-workers (446) reported that the translational repressor was a late gene product and instead postulated that gene 0.3 encodes the so-called M (membrane) protein (446), which was supposed to make the *E. coli* membrane more permeable, allowing an efflux of potassium ions and creating an optimal ionic environment for the synthesis and function of phage-induced enzymes (366, 367). Herrlich and co-workers postulated that this drop in ionic strength was the basis for the following functions of the M protein (366, 446): (i) killing of the host cell, possibly via inhibition of host macromolecular syntheses; (ii) protection of infecting T7 DNA against host nucleases of the *recBC* and restricting types; (iii) mutual and superinfection exclusion of T3 and T7; and (iv) alteration of phospholipid synthesis after T7 infection, resulting in the appearance of new polar lipids. None of these assumptions has been substantiated (see Shutoff of Host and Class I and Class II Phage Functions, *E. coli* Transfection, and The Problem of Heterologous and Homologous Superinfection Immunity). The true function of gp0.3, the blocking of restriction enzymes, is exerted not via a reduction of ionic strength, but rather through direct binding of the gp0.3 to the restriction enzyme (470).

Even the significance of the potassium efflux from the cell due to M protein action has been questioned. First of all, efflux had only been observed at subnormal (1 μM) potassium concentrations in the medium (366), and, furthermore, S. Michel and co-workers (personal communication) have observed no such passive K^+ loss from T3-infected cells. In addition, Michel and Brux (Abstr. Commun. Annu. Meet. Biochem. Soc. GDR, 11th, Halle/Saale, 1979, E14) (see Transcription) showed that, in the case of T3, transcription by phage RNA polymerase is carried out in vivo despite the presence of normal potassium concentrations, provided that a functional *E. coli dnaB* protein is present.

It is a fact that T7 gp0.3 is found associated with the cell membrane (120), but so are most other phage proteins, including even the major coat protein (gp10). McAllister and Wu (303) reported that neither deletions in the early T7 genome region nor a high concentration of monovalent cations (0.15 M KCl) had any effect on late T7 mRNA synthesis in vivo. Condit (79) observed a permeability change late in infection (beginning 16 min postinfection at 30°C) which is probably caused by gp3.5 (see Gene 3.5 Product and True Lysis Enzyme).

DNA REPLICATION, MATURATION, AND RECOMBINATION

Great progress has been made in the study of DNA replication, and several reviews have appeared to which we shall refer for general facts (8, 105, 132, 211, 236, 237, 314, 390, 459, 522, 545). DNA replication is initiated through a highly specific recognition step (for review, see references 8, 236, and 444), at a unique origin site(s), in some cases by incision of one of the parental DNA strands and in others by the synthesis of an RNA primer ("*ori*" RNA) from the origin sequence. DNA replication is catalyzed by a delicate multienzyme apparatus, the replisome (237, 522). Depending on the size and structure of the DNA template, different types of proteins are required for DNA strand separation (72, 211, 256). The replication fork opens up in advance of the growing "leading strand," which is probably synthesized continuously in 5′ to 3′ direction, whereas the "lagging strand" (3′ to 5′) must by necessity be synthesized in fragments, each initiating with a short RNA primer (343, 545).

DNA synthesis of double-stranded DNA phage proceeds either by a rolling circle mechanism or bidirectionally on linear or circular templates and produces characteristic intermediate DNA structures (105). The newly replicated DNA has to "mature," i.e., be processed, in order to yield native-sized progeny DNA (105, 390), and the ultimate stages of DNA maturation are coupled to phage morphogenesis.

Apart from the first round of replication, the synthesis of T7 DNA is spatially separated from the cellular region where parental T7 DNA is being transcribed. This might be achieved by different sites of membrane attachment of transcribing and replicating complexes (187). It is noteworthy that in the case of T7, DNA replication is not the precondition for late transcription (67, 484), as it is for T-even phage.

Enzymology of DNA Synthesis

Breakdown of host DNA. About 5 min after T7 infection (37°C), *E. coli* DNA is released (probably aided by gp3.5 [460]) from a fast-sedimenting structure and subsequently disrupted into fragments (2×10^6 daltons) by gene 3-specified endonuclease. These fragments are totally digested to deoxynucleoside monophosphates by the gene 6 exonuclease (61, 222, 223, 359, 414). T7 and T3 induce no nucleotide-synthesizing enzymes of their own and are thus fully depend-

ent on the precursors acquired by host DNA degradation. The two phage-coded nucleases (gp3 and gp6) are also involved in DNA recombination and concatemer formation (see below), whereas other apparently T7-specified endonucleases (57, 348, 411) have not been assigned any definite role in T7 DNA metabolism.

Primary initiation. The origin of replication (*ori*) on T7 DNA was placed at 17% by electron microscopy (106) and has now been mapped precisely by Saito et al. (418) as a 129-base-pair sequence between 14.73 and 15.05 map units. It spans the right half of the 14.75 promoter, a 61-base-pair noncoding intercistronic region, and the first 14 codons of gene 1.1. The intercistronic sequence contains seven copies of the palindrome TTAA, which would allow the formation of hairpins, and there is a gene 4 protein (primase) site, GACCC, within. Saito et al. assume that at this position leading-strand synthesis is initiated by the primase (see below) and proceeds rightward until the first primase site on the opposite strand is exposed. This initial rightward movement might explain why eye-form intermediates at position 17 are observed. Marker rescue studies after ultraviolet irradiation had also suggested that the origin of replication is situated to the left of 17% (45, 46). This major origin initiates 70% of phage DNA synthesis (388). Deletion mutants lacking this origin are apparently viable (462), and minor origins have been identified (388, 418, 435). The predominant secondary origin is located at approximately 4% (418), near promoter *B*.

The proximity of *ori* to class II promoters has been noted (106, 229, 230, 390), and it was proposed that T7 RNA polymerase plays a role in primary initiation. This is supported by Hinkle's observation that thermoinactivation of a temperature-sensitive T7 RNA polymerase causes a cessation of phage DNA synthesis (189). The function of T7 RNA polymerase in initiation is probably the transcriptional activation of the *ori* region (see Fig. 2), a process described in the review of Kolter and Helinski (236). A facultative role of gp1.1 and gp1.2 in primary initiation is assumed (418).

DNA polymerase. T7 DNA polymerase (DNA nucleotidyltransferase, EC 2.7.7.7) has an intriguing structure. It consists of two subunits (230): the phage gp5 (150, 322, 340) plus the product of the *E. coli tsnC* gene (321), which was identified as thioredoxin (292). Thioredoxin was originally isolated as the cofactor of ribonucleoside-diphosphate reductase (265). It is a protein of 12,000 daltons (292) with a very high cysteine content. In relation to its function in T7 DNA replication, it may be important that thioredoxin can be phosphorylated at cysteine-32 (80, 365). Native T7 DNA polymerase is a dimer of these two subunits (7, 322). These may be separately purified from T7-infected cells (197) lacking either thioredoxin (*tsnC* [*trx*] mutants [195]) or gene 5 protein, and, on reconstitution, they yield functional T7 DNA polymerase (7, 198).

T7 DNA polymerase displays 3′-5′ exonuclease activity (150, 340). gp5 itself is a single-strand-specific 3′-5′ exonuclease possessing a deoxynucleoside triphosphate binding site (7, 198) but nevertheless unable to catalyze even a single polymerization step (198). The reconstituted enzyme, besides catalyzing DNA synthesis, carries a 3′-5′ exonuclease activity for double-stranded DNA. It also has the single-stranded DNA 3′-5′ exonuclease activity of gp5 (7, 198). The relationship between structure and function of T7 DNA polymerase is reminiscent of the fact that the phage T4 DNA polymerase *amB*22 fragment (which lacks the cysteine-rich 20% COOH-terminal region) is devoid of polymerase activity but retains 3′-5′ exonuclease activity (334, 335).

Ribonucleic acid primer synthesis. Nascent T7 DNA fragments complementary to the heavy strand are linked to RNA (313). Discontinuous synthesis of one of the strands has also been demonstrated by electron microscopy (528, 529) and hybridization studies (476). RNA primers are synthesized by a multifunctional phage enzyme, the gene 4-coded primase (432, 439), essential in vivo for lagging-strand synthesis (476, 529). In vivo the primers are two to six nucleotides long and composed mainly of adenosine and cytidine; the first deoxynucleotide is deoxyribosylthymine monophosphate (342, 447). Primer structure in vivo is more diverse than that in vitro, where predominantly pppACCA and pppACCC are made (188, 402, 403, 433, 434), which are also the most effective primers for subsequent DNA synthesis. T7 primase has been purified and characterized (192, 233, 433, 434, 439, 476). It occurs in a 66,000-molecular-weight species (434) and a 58,000-molecular-weight species (233) (see Table 4) which display identical enzyme activities in vitro.

T7 primase has several copurifying activities (188): (i) single-strand-DNA- and (in the presence of T7 DNA polymerase) also double-strand-DNA-dependent hydrolysis of (preferentially deoxy)nucleoside triphosphates (234), providing the energy for (ii) catalytic participation in DNA strand separation (188, 433) as a "helicase" (see references 72 and 256), (iii) catalysis of RNA primer synthesis (432–434, 439), and (iv) stimulation of primer extension by T7 DNA polymerase (188, 402).

The native enzyme is a dimer of two identical

subunits (188). The intimate functional cooperation of T7 primase and DNA polymerase is revealed by their mutual sensitivities to each other's inhibitors: (i) dideoxythymidine triphosphate interrupts DNA chain growth and concomitantly stops gp4-catalyzed (deoxy)nucleoside triphosphate hydrolysis, and (ii) β-γ-methylenedeoxyribosylthymine triphosphate, a primase inhibitor, arrests DNA synthesis (234). Neither the three *E. coli* DNA polymerases nor phage T4 DNA polymerases are stimulated by T7 gp4 (434); thus, the interaction between T7 DNA polymerase and primase is highly specific. T7 primase can, however, cooperate with T3 DNA polymerase (188). In many respects the T7 primase-DNA polymerase enzyme pair is functionally equivalent to the *E. coli dnaB-dnaG* protein complex, acting as a "general priming system" for DNA replication (10, 237, 473).

RNA primers accumulate in T7-infected cells lacking gp6 and *E. coli* DNA polymerase I-associated 5′-3′ exonuclease (454). Purified gp6 displays RNase H activity (455), so gp6 is presumably responsible for primer degradation in vivo.

DNA-unwinding protein and DNA ligase. Whereas T7 DNA polymerase and primase are essential phage functions, T7 also induces a nonessential DNA-unwinding protein (the corresponding *dup* gene has not been precisely mapped as yet). The protein was purified and shown to specifically stimulate T7 DNA polymerase (385, 386, 438). In contrast to the specificity of the T7 DNA-unwinding protein, the analogous *E. coli* protein stimulates *E. coli* DNA polymerases II and III (holoenzyme) and also T7 DNA polymerase, accounting for the fact that *dup* protein is dispensable during T7 reproduction (323, 324). The properties of DNA-unwinding proteins (also termed helix-destabilizing proteins) were recently reviewed (72).

T3 and T7 gene 1.3 encode an adenosine 5′-triphosphate-dependent DNA ligase (293, 493) which can be substituted in vivo by *E. coli* ligase, although the latter is nicotinamide adenine dinucleotide dependent (21, 293). One may ask why T7 (and T3) encode proteins which are functionally replaceable by host proteins. The explanation is probably that the DNA-unwinding protein is needed in stoichiometric amounts and that DNA ligase must seal DNA fragments at a multitude of sites simultaneously, also necessitating a high enzyme concentration.

DNA-relaxing enzymes. DNA gyrase (topoisomerase II), an enzyme with the capability of relaxing and introducing superhelical turns, is essential for DNA replication in *E. coli* (see reviews by Jovin [211] and Champoux [72]). In addition to topoisomerase I (ω protein), two types of DNA gyrase (topoisomerases II and II′) are present in *E. coli* which differ functionally as well as in the size of the smaller subunit (41). This subunit is encoded by the *gyrB* (*cou* [coumermycin resistance]) gene of *E. coli* and the larger subunit is encoded by the *gyrA* (*nalA* [nalidixic acid resistance]) gene (72). Itoh and Tomizawa (208) showed that coumermycin inhibits T7 DNA replication in *E. coli* wild type and concomitantly less in coumermycin-resistant host cells, indicating that at least the *gyrB* (*cou*) gyrase subunit is involved in phage DNA replication. DeWyngaert and Hinkle (97) confirmed these in vivo results but showed that gyrase is not required in vitro. Coumermycin also inhibits late T7 transcription (97).

The requirement for topoisomerases during the replication of circular double-stranded DNA is evident; the apparent role of DNA gyrase during T7 phage DNA replication (208) was explained by the collapsed state of replicating T7 DNA in vivo (95) or by its membrane attachment (97, 208), either severely limiting free rotation of the linear double strands. The validity of this interpretation has recently been challenged by Kreuzer and Cozzarelli (242), according to whom nalidixic acid inhibits the growth of T7 phage, whereas heat inactivation of a thermosensitive *nalA* (*gyrA*) gyrase subunit does not. They infer that nalidixic acid blocks T7 growth by a corruption of the enzyme which, per se, is not necessary for T7 DNA synthesis. This corruption could, for instance, consist in trapping of a gyrase reaction intermediate (495) and would explain the dominance of drug susceptibility in vivo (242). In this connection it is remarkable that certain phage T3 DNA polymerase (gene 5) *ts* mutations confer a partial nalidixic acid resistance to T3 replication in vivo (443). In the light of Kreuzer and Cozzarelli's work, it is warranted to reevaluate the effects of coumermycin on T7 DNA replication.

Reconstituted in vitro systems. Attempts to reconstitute T7 DNA replication in vitro date back to 1973 (230), and partially reconstituted systems containing T7 DNA polymerase and primase with or without DNA-binding protein were described later on (191, 432). Replication forks formed in vitro closely resemble those formed in vivo (31, 432). Masker and Richardson (296, 297) developed an in vitro system including *E. coli* DNA polymerase I which produces mature-size T7 DNA capable of transfecting *E. coli* spheroplasts. Richardson et al. (388) have succeeded in reconstituting an enzyme system which performs primary initiation on intact duplex T7 DNA (see above) and carries out leading- and lagging-strand synthesis at the replica-

tion fork in a manner similar to that observed in vivo. Thus, the reactions occurring at the replication fork are now thoroughly understood (see Fig. 4). However, the role of the replicative intermediates described in Structure of Replicating and Maturing DNA is still not clear.

Structure of Replicating and Maturing DNA

T3 and T7 DNAs possess terminal redundancies (391) which are essential for phage multiplication (22, 104). The length of the terminal repetition of T7 DNA has now been estimated by independent methods to be 150 to 160 base pairs (103, 405). Due to unidirectional DNA polymerase movement, unit-size DNA has no means of replicating the 3'-terminal gaps which arise by excision of primer RNA. Watson (520) therefore postulated that linear duplex DNA must replicate via concatemers which form by hybridization of the unreplicated 3' redundant regions of newly synthesized DNA. To this day, Watson's postulate has not been conclusively proved or disproved.

The first round and at least the second round

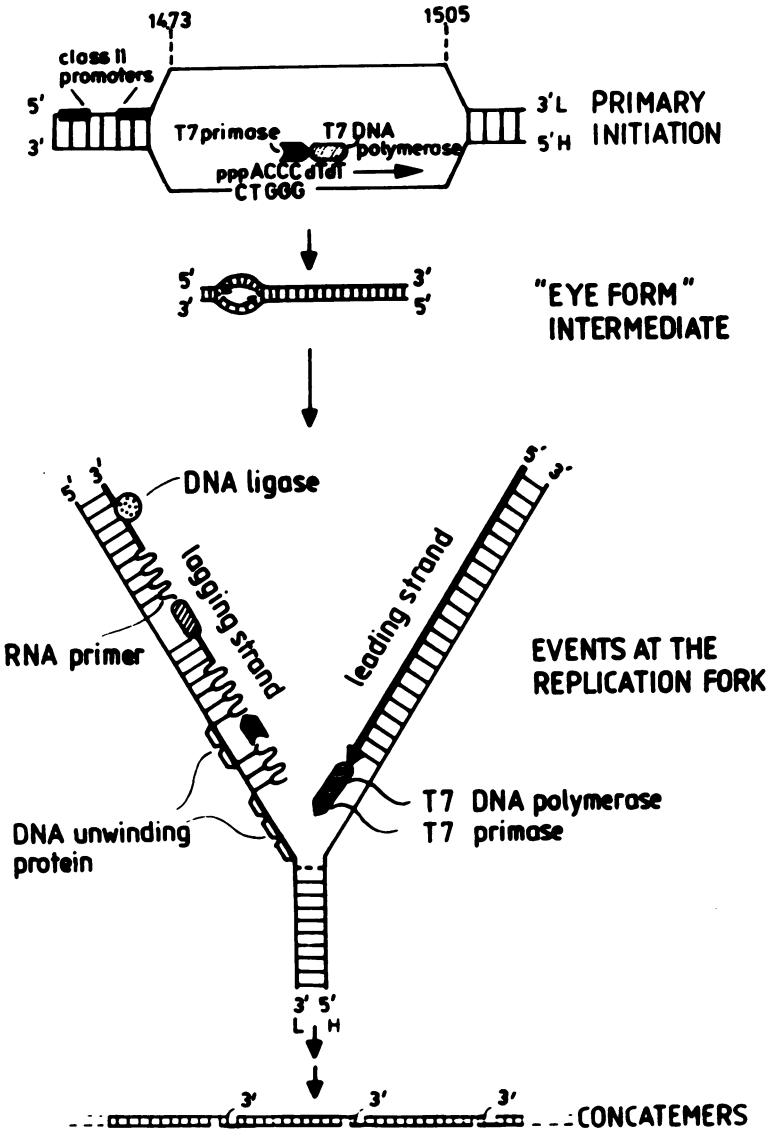

FIG. 4. *Stages of phage DNA replication. For explanations, see text. L, Light strand; H, heavy strand.*

of T7 DNA replication begin at the *ori* site (see above) and proceed bidirectionally, creating first an eye and later on a Y-shaped intermediate structure (106, 528, 530); no circles are formed 530) (see Fig. 4).

Intracellular T7 DNA is associated with the membrane. (For technical details of studying phage DNA-membrane interactions, please consult the review of Siegel and Schaechter [459].) At a few minutes postinfection, parental T7 DNA is found associated with the inner cell membrane (also in the absence of phage protein synthesis) (58, 59, 347). The membrane complex contains phage DNA polymerase and actively synthesizes DNA (59); this DNA synthesis is just as ultraviolet sensitive as T7 in vivo DNA replication (526). Apparently, parental and progeny DNA molecules are bound to the membrane with different strengths (187). Parental DNA remains attached to the membrane throughout infection (187)—the attachment site is located within the early DNA region (175)—whereas replicating DNA is eventually released from the membrane (450). Parental DNA seems not to participate in concatemer formation (126). Since T7 DNA replication in vitro does not require membrane components, membrane association seems to serve an organizational role in vivo.

Concatemer DNA in T7-infected cells was initially observed by Hausmann (162), Carlson (53), Kelly and Thomas (221), Hausmann and LaRue (169), and Ihler and Thomas (204). Later during replication, there appear condensed coiled progeny DNA structures (30, 60, 187, 295, 350, 372, 440, 450, 476, 477, 510), which are also seen in T3-infected cells (129). This complex DNA was purified and characterized (260, 350). It contains about 100 phage genome equivalents and sediments at approximately 440S. In contrast to the compact folded *E. coli* genome (531), the T7 DNA complex is not held together by RNA, but contains hydrogen-bonded unit-size phage DNA (126, 440, 450, 477). Concatemer formation does not require recombination and may proceed when both the gene 3 and the gene 6 nucleases are simultaneously absent (126, 315). Under normal circumstances gp6 is essential for T7 (126, 169, 315) or T3 (539) concatemer formation. gp3 of T7 is not essential for in vivo and in vitro concatemer formation (295, 315, 415). The apparent requirement of gp3 for T3 DNA concatemerization probably reflects the polar effect of a gene 3 amber mutation on the expression of the *dup* gene. T3 DNA-unwinding protein may function in concatemer formation in vitro and also in vivo (129). T7 gp2 is required for the maintenance of concatemer structure (60).

The pathway of DNA replication and maturation is conceived to be as follows: unit-size DNA → short concatemers → complex fast-sedimenting intermediate → short concatemers → unit-size DNA (350). Net T7 DNA synthesis is completed at 17 min postinfection at 30°C (260). The rapid, site-specific cleavage of concatemers into monomers is performed at the junctions between adjacent monomers, after the repair of gaps or protruding "whiskers" at these junctions (440). This is in accord with the above-mentioned postulate of Watson (520). The intimate connection between DNA maturation and packaging is discussed in Phage Morphogenesis and Cell Lysis.

DNA Recombination

Molecular recombination is inherent to, but not necessarily essential for, T3 and T7 development and is coupled to the most active phase of DNA replication (294, 372). Recombination in vivo requires gp3, gp4, gp5, and gp6 (225, 371). Recombining DNA is fragmented before recombination (415), and gene 6 exonuclease is necessary for breakage and reunion of the fragments in parental-to-progeny DNA recombination (269, 315, 508). In the case of cloned DNA fragments, recombination into T7 DNA does not require gp4, gp5, and gp6 (51).

Biparental recombining T7 DNA molecules adopt characteristic intermediate structures in vivo (H- and Y-shaped) in a gp6-catalyzed reaction in the absence of gp3 (510). These branched structures are stabilized by gp5 (which plays a role other than gap filling) and are converted to linear recombinant molecules by the action of gene 3 endonuclease I (510). The same intermediates as in vivo arise in extracts of T7-infected cells (341).

Sadowski and Vetter (415) devised an in vitro assay which measures recombination of exogenous mature T7 DNA with endogenous T7 DNA present in extracts of infected cells. These extracts promoted recombination as well as packaging, so the recombinants could be assayed as plaque-forming units. This biological assay was complemented by a (less sensitive) physical assay (514) measuring the density shift of labeled exogenous phage DNA. It became clear that polar packaging (see Phage Morphogenesis and Cell Lysis) simulates asymmetric recombination in the biological assay (393), so Roeder and Sadowski (399) developed mutually independent and noninterfering in vitro T7 DNA recombination and packaging assays, proving that recombination is not inherent to in vitro packaging. This new recombination system, designed to promote recombination between exogenous DNA molecules, has permitted at least two pathways of in vitro recombination to be distin-

guished in appropriate mutant extracts: the endonuclease pathway, dependent on gp3, gp5, and either host or phage (gp1.3) ligase and inhibited by gp4 plus deoxynucleoside triphosphates, and the exonuclease pathway, requiring gp6, independent of ligase and gp3, gp4, and gp5, and stimulated by deoxynucleoside triphosphates. The wild-type in vitro pathway seems to combine features of both these separable pathways (399). As to enzyme requirements, the main difference between the in vitro and the in vivo reactions is the dispensability of gp4 and gp5 in vitro. Roeder and Sadowski (399) suggest that in vivo the physical separation of parental DNA molecules (bound to different membrane sites) may exclude biparental recombination so that only replicating DNA can recombine. On the other hand, according to the Radding-Meselson model (199), the products of genes 4 and 5 may be involved in DNA synthesis at nicks or gaps. Electron microscopy of recombining T7 DNA suggests that recombination is primed by an invasion of single-stranded DNA into single-stranded gaps at the replication fork (372).

In vitro recombination systems will probably soon allow the complete fractionation, reconstitution, and functional characterization of the individual recombination factors.

PHAGE MORPHOGENESIS AND CELL LYSIS

T3 and T7 Assembly

In vitro packaging. In vitro packaging is an approach to resolve the steps of phage assembly and is also a useful procedure for assaying in vitro-manipulated DNA (see DNA Recombination). By heat-inactivating mature phage, Serwer (451) obtained a T7 DNA-nucleocapsid structure consisting of a unit length of T7 DNA to which an empty phage head is attached. Similar structures were seen in lysates of T7-infected cells. In the in vivo structure the attachment site was 7% from the genetic right end of the DNA; the detection of DNA-head complexes containing double-length DNA indicated that head binding is involved in the ultimate DNA maturation step (451).

Kerr and Sadowski (224) developed a combined in vitro DNA recombination and packaging assay. To study packaging uninfluenced by DNA recombination, Kuemmerle and Masker (255) introduced the use of gene 3, 5, and 6 triple mutants. Concatemeric DNA is packaged 10^4 times more efficiently than unit-size DNA (295), indicating that concatemer formation is a prerequisite for DNA packaging in vivo. In vitro packaging depends on the presence of the following components (413): proheads (400); the proteins coded by genes 18 and 19, which are necessary for head filling (224, 400); and the tail proteins gp11, gp12, and gp17.

Fujisawa et al. (128, 319) developed an analogous in vitro packaging system for T3 DNA. Mature T3 DNA is converted to concatemers by the combined action of gp3 and gp6 and packaged into proheads in the presence of gp4, gp5, and gp19.

In vivo morphogenesis. The basics of virus morphogenesis have been reviewed (55, 326). Since the 1976 review of Hausmann (165), a number of new papers dealing with T7 and T3 morphogenesis have appeared (117, 128, 207, 319, 400, 449, 452, 453, 534), of which we shall present a brief summary.

The proteins gp8, gp9, gp10, gp13, gp14, gp15, and gp16 assemble into proheads, and in the presence of gp18 and gp19 plus DNA, packaging is initiated. gp9 is a scaffolding protein necessary for the formation of the packaging intermediate but no longer present in the mature phage particle. Heads which have initiated, but not completed, DNA packaging are unstable and dissociate into DNA and empty heads. The final DNA maturation steps are synchronous with the completion of packaging. Somewhere between prohead formation and the completion of packaging, a host factor is active. Full heads do not contain gp18 and gp19.

gp11 and gp12 attach to full heads, forming the conical tail to which the tail fibers (gp17) are subsequently bound.

Mature phage heads consist mainly of gp10. In addition, gp14, gp15, gp16, and, probably, gp13 form a core which is surrounded by DNA and extends into the tail. This core structure might constitute an initiation complex during capsid assembly. gp8 is located at the junction of the phage head, core, and tail. gp7 and gp13 are not necessary for assembly, but they are necessary for the infectivity of the mature particle. All maturation steps proceed without demonstrable proteolytic cleavage.

For the proportions of the individual T7 structural proteins within the mature phage particle, see Hausmann (165).

The ultimate DNA maturation and packaging steps are blocked in T7 gene 2 mutants (96, 267, 344). gp2 is essential for concatemer processing (267, 344). This requirement appears to involve inhibition of *E. coli* RNA polymerase by gp2 (as described in Shutoff of Host and Class I and Class II Phage Functions), inasmuch as gp2 is replaceable by rifampin in vivo (344) and in vitro (267) and as *E. coli tsnB* mutants which induce a gp2-tolerant RNA polymerase also impose a

barrier on late T7 DNA replication (see *tsn* Mutations of *Escherichia coli*). On the basis of published data it is conceivable that an immobile gp2-RNA polymerase complex firmly attached to promoter *E* (92% on T7 DNA [see Table 1]) serves as the landing base for the phage prohead. This position corresponds exactly to that observed by Serwer for the binding of empty heads to T7 DNA (451). The contact point on the prohead might be gp10. If this were so, many isolated observations concerning the role of *E. coli* RNA polymerase and phage gp2 and gp10 would fall into place. Host mutants unable to support T7 DNA maturation, due to a disturbed interaction between host RNA polymerase and gp2 (96, 267), map in *rpoB*, the structural gene for the RNA polymerase β subunit. The *E. coli* Y49 mutant selected for its inability to plate T7 0.7 (protein kinase)-negative mutants, imposes a similar block on phage maturation (534, 535). It is probable that the Y49 mutation, like the BR3 mutation described by Studier, which is also nonpermissive for gene 0.7 mutants (485), maps in *rpoB* as well. An abortive packaging reaction is also observed in *S. sonnei sin* (see Influence of Host-Specific Factors on Virus Multiplication) which can be overcome by a mutation in T7 gene 10 (513). *sin* may be yet another *rpoB* mutation. If this explanation is correct, then there is in fact only one host factor involved in phage T7 maturation, the bacterial RNA polymerase.

Gene 3.5 Product and True Lysis Enzyme

The role of the gp3.5 ("lysozyme"), an *N*-acetylmuramyl-L-alanine amidase, has been debated for several years (93, 127, 205, 210, 228, 484). This enzyme was initially thought to be responsible for cell lysis (180, 205). Studier (484) already pointed out that gp3.5 is expressed among the class II genes whose products function in DNA metabolism and, furthermore, that T7 gene 3.5 mutants are capable of inducing cell lysis. Jensen and Pryme (210) demonstrated that the standard test involving the liberation of radioactive material from diamino[^{3}H]pimelic acid-labeled *E. coli* cell walls (445) is no proof of cytolytic potency in vivo and identified an additional amidase activity in T7-infected cells. The latter is responsible for cell lysis (210). Miyazaki et al. (320) and Blair et al. (cited in reference 25) showed that the true lysis enzyme is encoded by a class III gene in T3 and T7 located somewhere between genes 17 and 19 (gene 17.5?).

gp3.5 functions in DNA metabolism and probably promotes the release of host and newly made phage DNA and phage particles from the cell membrane (460). It also seems to be engaged in the shutoff of class II mRNA synthesis (303).

Since the designation "lysozyme" is associated with active disruption of the cell wall and cell lysis, it would be sensible to abandon this name for the gene 3.5 product. To avoid confusion between gp3.5 and gp17.5, we propose to call the latter the lysis enzyme.

The lysis enzyme destroys the cell and liberates progeny phage particles. However, even in its absence, cell destruction is bound to occur eventually as the result of extensive phage-mediated cell damage (see Regulation of Gene Expression).

INFLUENCE OF HOST-SPECIFIC FACTORS ON VIRUS MULTIPLICATION

The study of bacteria which have lost or attained the ability to support virus growth is often very useful for defining the role of certain bacterial elements in phage development and complements the investigation of phage mutants. In the course of this review we have mentioned a number of host proteins which are active during different stages of the phage growth cycle: the *E. coli* RNA polymerase and thioredoxin as the most important of these factors; the *dnaB* protein, which is essential for late T3 transcription; and an as yet ill-defined host factor possibly involved in a late morphogenesis step (534, 535). In the following section we will discuss the roles of certain host factors in greater detail.

tsn Mutations of *Escherichia coli*

The isolation of *E. coli tsnB* and *tsnC* mutants by Chamberlin (67) was of great importance for T7 research. The further study of the *tsnC* mutation led to the fundamental discovery that the *tsnC* gene is the structural gene for thioredoxin (*trx*) and that *E. coli* thioredoxin is a necessary constituent of T7 DNA polymerase (see Enzymology of DNA Synthesis).

tsnB mutations map within the structural gene for the β subunit of *E. coli* RNA polymerase (*rpoB*) (see review by Yura and Ishihama [543]). Investigations of *tsnB* mutants and of another *rpoB* mutant, *E. coli* BR3, which is unable to sustain the growth of T7 gene 0.7 mutants (485), proved that *E. coli* RNA polymerase is indispensable not only for early phage transcription but also for a late DNA maturation step (see Structure of Replicating and Maturing DNA) (96, 98, 267).

In an F^+ background, nonpermissive for T7 reproduction (see below), the introduction of *strA* mutations (affecting ribosomal protein S12) allows T7 phage to overcome the F^+ barrier. The

original, nonpermissive state can be fully restored by an additionally introduced *rpoB* (rifampin resistance [*rif*]) mutation (62, 63). A survey of *rpoB* mutants of different phenotypes as to their effects on phage replication would probably greatly further our understanding of T7 virus-host interaction.

Interference by F Plasmids

T7 infection of host cells harboring an F plasmid is abortive, and T7 is, therefore, termed a "female-specific" phage (286). After initial results according to which two adjacent F-factor genes, *pifA* and *pifB*, determine an obstruction of late T7 mRNA translation, a number of papers described membrane permeability changes and a cessation of macromolecular syntheses ahead of late translation (for references, see Condit [78] and Hausmann [165]). Recently, Remes and Elseviers (384) proved that adenosine 5'-triphosphate leakage is not causally related to T7 exclusion. Despite numerous attempts to solve this problem, the ultimate cause of abortive development and the respective roles of chromosomal, plasmid, and phage genes in T7-infected F^+ cells are not completely clear. The F-plasmid genes responsible for T7 exclusion were recently cloned (465) and shown to be localized at the 38.3 position on the F-plasmid map (353).

Schell et al. (429) and Williams and Meynell (524) had reported an F-mediated exclusion of T3, whereas Hausmann (163) and Studier (490) failed to detect such a phenomenon. This discrepancy is explained by the fact that the phages used by the former authors were actually T7 strains (490). T3 can not be termed "female specific." It is to be hoped that a comparison of T3 and T7 will lead to the identification of the phage function(s) which confer resistance and sensitivity, respectively, to F^+ *pif* gene action.

P1 Prophage

The impaired growth of T3 and T7 on P1-lysogenic cells has been known for a long time (122, 138, 268). Hausmann noted that gene expression declines sharply from the early to the late genes and that this drop occurs more rapidly in the case of T7 (163).

Krüger and co-workers (251; D. H. Krüger, S. Hansen, M. Reuter, and C. Schroeder, manuscript in preparation) have shown that the block on T3 and T7 development is caused by the restriction enzyme *Eco*P1. However, no cleavage of T3 or T7 DNA in P1-lysogenic cells is observed. It was proved that T3 (sam^+), compared with T7 and T3sam^- mutants, has a superior ability to express its genes and damage the cell. Restriction endonuclease *Eco*P1 molecules appear to bind to T3 and T7 DNAs and, similarly to repressors, inhibit transcription. DNA cleavage is prevented by the phage ocr^+ function. Due to the expression of SAMase and the resulting lack of SAM in the T3-infected cell, the repressor-like binding of the P1 endonuclease is less stable. T3 and T7 ocr^- mutants are subject to in vivo DNA cleavage and methylation by restriction endonuclease *Eco*P1. The investigation of in vivo restriction of phages T3 and T7 by *Eco*P1 has aided the dissection of the different levels on which the sam^+ and ocr^+ functions influence the interaction between DNA and restriction enzymes (see Interactions with the DNA Modification and Restriction System of the Host: Functions of Gene 0.3).

It is known that *Eco*P1 and *Eco*P15 show differences from the type I restriction enzymes in their in vitro properties (11, 151, 542) and, moreover, recognize a shorter nucleotide sequence (16, 152)—which should occur with a higher probability within a given DNA molecule—than does *Eco*B or *Eco*K (214, 264, 381, 468). It is still not clear which special feature of T3 and T7 is responsible for the strong repressor-like effect of the P1 restriction enzyme in vivo. In contrast to the drastic in vivo effect of *Eco*P1 on T3 and T7 DNAs, phage lambda and the plasmid pSF2124 DNAs are restricted by *Eco*P1 in vivo in the usual manner, which also obtains for the restriction of lambda and ocr^- derivatives of T3 and T7 by *Eco*B and *Eco*K (no repressor effect) (11, 254, 387). In addition, *Eco*P1-mediated restriction of pSF2124 DNA can be completely overcome by ocr^+ activity (387).

Antibiotic Resistance Plasmids and Colicinogenic Plasmids

Some older papers describe the inhibition of T3 or T7 phage growth by certain R plasmids (18, 312, 519). In none of these cases was the mechanism of exclusion (e.g., adsorption, abortive infection through incompatibility, DNA restriction) elucidated.

Our preliminary studies on the influence of the restriction endonucleases *Eco*RI and *Eco*RII on the propagation of T3 and T7 in vivo show that these restriction systems do not significantly inhibit virus growth (Krüger, unpublished data). This is not surprising in the case of *Eco*RI, since T3 and T7 DNAs have no recognition sites for *Eco*RI (17, 260, 405, 421). The DNA M/R system of plasmid R124, which has also been termed *Eco*RIII (464) and which corresponds to a type I restriction enzyme (S. G. Hughes, personal communication), does not restrict T3 phage, on account of the protective effect of the

ocr$^+$ gene function; T3*ocr*$^-$ mutants are restricted and modified in vivo (D. H. Krüger, S. Hansen, and L. S. Chernin, manuscript in preparation).

Various colicinogenic plasmids interfere with T3 or T7 growth not on the level of adsorption or DNA restriction, but in an abortive infection through incompatibility (146, 168).

Shigella sonnei D_2 371-48

The infection of *S. sonnei* D_2 371-48 by T7 phage ends abortively. Though DNA synthesis begins as usual, it is followed by extensive phage DNA breakdown for which a suicidal (wild-type) phage function *ss*$^+$ is responsible, since T7*ss*$^-$ mutants are capable of normal growth in the *Shigella* strain. During infection of *E. coli* host cells, the *ss*$^+$ phage function is compensated by a "suicide inhibition function" of the cell (*sin*$^+$), whereas *S. sonnei* D_2 is *sin* (cp. 164, 165). The *ss* marker was recently localized within the gene coding for the major head protein (gene 10) (513). The fact that a gene 10 mutation counteracts the suicidal behavior that normally occurs upon infection of *S. sonnei* D_2 (*sin*) suggests that an interaction between a host function (*sin*$^+$) and gp10 of T7 may normally take place during phage head morphogenesis. The abortive infection of *S. sonnei* D_2 is caused by the block of phage assembly; the degradation of T7 DNA is a secondary effect (513). T3 growth is unperturbed on *S. sonnei* D_2 (203); T3 thus behaves as a "natural" *ss*$^-$ mutant. This is corroborated by the apparent nonhomologies between the gene 10 base sequences of T3 and T7 (89, 203).

Because of the facile discrimination between *ss*$^+$ and *ss*$^-$ T7 phage with the help of *S. sonnei* D_2, these markers have been used as experimental tools for in vitro DNA recombination and packaging (294, 399, 413).

SPECIAL VIRUS-VIRUS AND VIRUS-HOST INTERACTIONS

T3 as a Facultative Temperate Phage

Lysogeny of bacterial cells has been studied in detail in the cases of such temperate phages as lambda and P22 (19, 118, 181, 500). The most important criteria for lyzogenization are the perpetuation of prophage as part of the bacterial replicating system and the ability of the lysogenic cell to produce progeny phage (prophage induction) without reinfection. Phage-coded repressor molecules prevent the expression of lytic phage functions and are also responsible for the superinfection immunity of the cells to homologous phage. Due to the intracellular state of the phage, it is resistant to phage antiserum treatment. In addition to lysogeny, there are other less intimate virus-cell interactions, called pseudolysogeny or carrier state, which have not been strictly defined (19, 125, 174, 209, 278, 505, 518).

In the 1950s Fraser and co-workers (124, 125, 182) noticed that after infection by phage T3 of *E. coli* cells starved in buffer, there is no immediate phage replication and special so-called late-lysing complexes are formed. In 1972 Krüger et al. (252) observed that upon infection of starved *E. coli* B cells, a fundamental difference between T3 and T7 becomes evident. Whereas, T7 induces a normal lytic cycle, the T3 genome forms a complex with the cell. During slow cell growth in minimal medium the phage genome is coreplicated, and the daughter cells carry the viral information. A transfer of T3-lysogenic cells into rich medium causes induction of T3 prophage, and a complete lytic cycle is performed. The facultative temperate behavior is connected to the T3 SAMase (*sam*$^+$) gene (243). Apparently the T3 genome, which is nonmethylated due to SAMase action during the preceding growth cycle (see Interactions with the DNA Modification and Restriction System of the Host: Functions of Gene 0.3), cannot be expressed in starved cells. In contrast, SAMase-negative phage with methylated genomes (T7, T3*sam*$^-$) are expressed in starved host cells. When, however, T3*sam*$^-$ phage are propagated under conditions where DNA methylation is prevented by preinfection with SAMase-proficient phage, these unmethylated *sam*$^-$ phage also establish a temperate interaction upon subsequent infection of starved cells (243). It is still a matter of speculation why the degree of methylation is so important for the behavior of T3 in starved host cells but not in normal cells. During the lysogenic state, not even the leftmost portion of the T3 genome is expressed (D. H. Krüger and C. Schroeder, unpublished data). Replication of the T3 prophage is thus under exclusive control of the *E. coli* replication system. Incubation of T3-lysogenic cells in minimal medium plus T3 antiserum has no influence on the lysogenic state; i.e., reinfection (carrier state) cannot be the cause for T3 perpetuation.

In contrast to authentic lysogeny, e.g., by phage lambda, T3 lysogeny is unstable and not maintained by phage-coded repressor action. The absence of a phage repressor is also manifested in the lack of superinfection immunity (Krüger, unpublished data). Up to now it is not clear whether T3 prophage is incorporated in the *E. coli* chromosome or whether it is "inherited" as a plasmid.

Thus, under special circumstances the normally virulent phage T3 can obey criteria of temperence. We prefer to call this type of virus-host interaction *lysogeny* to emphasize the abil-

ity of the growing cell to lyse after induction of provirus. A decision for a certain term would demand a more detailed knowledge of the underlying molecular processes. Possibly, *intracellular persistence* would be more appropriate, since the term *lysogeny* is traditionally associated with high stability and the expression of at least one phage gene product, the repressor protein. In its dependence on the phage and possibly the methylation degree of its genome, on the one hand, and the physiological state of the host cell, on the other, temporary T3 lysogeny provides an interesting system for studying the regulation of host "rescue" gene expression programs (e.g., the stringent control pattern [130, 329] or the SOS pattern [527]) on the level of transcriptional control and their possible relation to DNA methylation. First indications of a link between differentiation processes and DNA methylation have also been reported for eucaryotic systems (4, 75, 308, 501, 547).

E. coli transfection

The utilization of transfection studies on *Enterobacteriaceae* for the solution of different problems of molecular biology was recently reviewed by Benzinger (28). Progress in the transfection of *E. coli* spheroplasts by T7 DNA has been made by the groups of Benzinger (29, 176, 265) and Wackernagel (104, 448, 517). Ehrlich et al. (119) studied the transfection of $CaCl_2$-treated *E. coli* by T7 DNA.

T7 DNA is infective in the double-stranded and the single-stranded state (265, 297, 448, 517). Transfection by T7 single-stranded DNA was observed to depend on a functional DNA polymerase III in *polAB* cells, whereas transfection with native T7 DNA was independent of host DNA polymerase (448). The infectivity of double-stranded DNA in vivo is significantly reduced by the *recBC* nuclease, probably by an attack on the free ends of the linear duplex (29, 119, 448, 517). An in vitro erosion of the 5′ ends of these duplexes, i.e., the creation 3′-terminal single strands, does not lower, but rather enhances, infectivity (104, 119, 517), since these are poorer substrates for the *recBC* enzyme, and, furthermore, circularization of "terminally eroded" DNA takes place within the cell, making it completely insensitive to *recBC* digestion (104, 448, 517). However, when both strands of the terminal redundancies have been degraded in vitro, the DNA loses its infectivity. This is yet another proof of the essentiality of the terminal redundancies for DNA replication and maturation (104).

Transfection efficiency of restriction-proficient cells by T7 DNA is substantially reduced compared with restriction-deficient cells, which is explained by restriction of the entering DNA molecule (119). The fact that the *E. coli* strain used for propagating the phage from which the DNA was extracted had no influence on the degree of restriction (119) is explained by the fact that ocr^+ action prevented DNA modification (245). During normal infection, incoming DNA is protected against restriction by the ocr^+ function (see Interactions with the DNA Modification and Restriction System of the Host: Functions of Gene 0.3), but in the transfected cell this phage function is, of course, expressed too late. Interestingly, the 1,000-fold reduction of transfection efficiency of T7 DNA by restriction endonuclease *Eco*K (119) corresponds exactly to the drop in efficiency of plating of T7ocr^- mutants when they are plated on *E. coli* K (254).

Analogous to the fact that the DNA restriction system of the host does not inhibit T7 growth upon normal infection but upon transfection, the *recBC* nuclease seems only to interfere with transfection, and not with normal T7 infection (517). Phage whose DNA goes through a *recBC*-sensitive stage in their life cycle have evolved protective measures against the enzyme (28). This protection has been most thoroughly elucidated in the case of the gamma protein of phage lambda (216, 420). Sakaki (419) found that besides lambda and other phages, T3 and T7 are also capable of inactivating the *recBC* nuclease and that inactivation depends on phage gene expression. Wackernagel and Hermanns (516) came to the conclusion that the anti-*recBC* effect is caused by a late T7 protein. This protein was isolated and partially characterized (349). Unfortunately, no mutants in the corresponding gene are known.

Problem of Heterologous and Homologous Superinfection Exclusion

Hausmann et al. (171, 173) described the effect that in homologous (T3 × T3 and T7 × T7) crosses, the phage are compatible and both parental genomes are replicated and expressed, but heterologous mixed infection by T3 plus T7 results in mutual exclusion and only 5% of the mixed-infected cells produce both phage species. Beier (Ph.D. thesis) found that upon simultaneous infection, exclusion only takes place in the heterologous system, whereas upon superinfection, such an exclusion was postulated to occur in heterologous as well as homologous infection. The marker determining exclusion appears to reside in gene 1 (phage RNA polymerase) (Beier, Ph.D. thesis), indicating a role of the different template specificities of the phage RNA polym-

erases. Superinfection experiments with T3- and T7-preinfected cells confirmed the role of gene 1 (299), and the results of McAllister and Barrett (Table 1 of reference 299) let us conclude that gene 0.3 cannot be involved to any extent. Benbasat et al. (27) claim that after T7 infection, all superinfecting phage are excluded, since they adsorb but do not inject their DNA; this effect is supposed to be completely reversible by chloramphenicol; i.e., it is supposed to require protein synthesis by the first infecting phage. Hirsch-Kauffmann et al. (193) express the most extreme opinion. According to their results, T3 and T7 exclude each other in homologous as well as heterologous co- and superinfections. Only one phage genome should be able to enter the cell and block the entry of any other phage in a step subsequent to adsorption. This exclusion is allegedly mediated by gene 0.3 (called "M" gene by these authors [see Other Functions Ascribed to Gene 0.3]), without a requirement for RNA or protein synthesis (no chloramphenicol effect) directed by the excluding virus. Due to the postulated exclusion, no ultraviolet multiplicity reactivation of T3 and T7 should occur (193); however, multiplicity reactivation has been observed by other authors (45, 46).

As contradictory as these data are (Exclusion only on superinfection or also in coinfection? Exclusion only between heterologous or also between homologous phage? Role of gene 0.3 or gene 1 for exclusion? Necessity of gene expression of the first phage for the exclusion of all following phage?), they are as a whole opposed to quite self-evident facts of the molecular biology of T3 and T7. It is a well-known fact that amber mutants (23, 166, 483) and temperature-sensitive mutants (253, 442) efficiently complement each other in coinfected cells and that complementation is also observed in heterologous mixed infections (168). Obviously, complementation depends on the expression of at least two different phage genomes in the cell. The same is true for recombination experiments. Recombination frequencies in homologous crosses are normal (23, 483, 485, 494, 508). The recombination frequencies in heterologous T3 × T7 crosses are indeed lower than in homologous crosses (171; Beier, Ph.D. thesis), but the reason for this is that recombinants resulting from one (or any uneven number of) recombinational event are not viable and therefore not detectable, because recombinant DNA molecules carrying nonidentical terminal "redundancies" cannot form concatemers (22, 89, 104). (During experiments leading to the establishment of recombinational maps of T3 and T7, reproducibility is improved if phage infection is synchronized by applying KCN. This measure has no influence on the expression of gene 0.3 or 1.)

Bräutigam and Sauerbier (40) found a gene dosage effect depending on the multiplicity of infection of ultraviolet-irradiated T7 gene 1 mutants. Other authors found that net T3 DNA synthesis increases with the multiplicity of infection by T3 wild type (W. Mann, H. Musielski, I. Scheiber, and I. Laue, Abstr. Commun. Annu. Meet. Biochem. Soc. GDR, 11th, Halle/Saale, German Democratic Republic, 1979, B3). In connection with other experiments, Krüger and coworkers have shown that, for example, after preinfection of *E. coli* with T3 gene 4 or gene 6 amber mutants, subsequently infecting T3*sam*$^-$ normally replicates (243) and that preinfection by ultraviolet-irradiated T3 or T7 phage allows normal growth of T3/R7 and T7/D111 (245) and T3 and T7 wild types (Krüger, unpublished data). In all these cases a normal expression of gene 0.3 (and in the case of the amber mutants, also of gene 1) of the preinfecting phage takes place. All these results, including the complementation and recombination mapping data, imply that homologous or heterologous co- or superinfection immunity does not exist.

Since all experiments relating to this matter have been performed with phage mutants or ultraviolet-irradiated phage, there is, of course, the possibility that some kind of exclusion exists between wild-type phage. If there is a certain degree of mutual exclusion in the case of heterologous T3 × T7 crosses (171, 173), then it is certainly not related to gene 0.3 function.

The arguments given above hold only for simultaneous infection or for superinfection involving a delay of maximally 5 min (37°C). It is evident that a secondary infection after a longer lapse of time is bound to be unsuccessful because the processes in the infected cell are out of control.

Interactions with Eucaryotic Systems

Prompted by the spectacular finding of Merril and co-workers (134, 311) that λp*gal* induces galactose-1-phosphate uridylyltransferase in galactosemic human cells and by successful phage transduction of *E. coli* genes into plant cells (101), the interactions between phage and eucaryotic systems attracted the interest of molecular biologists (cp. 309, 310).

Up to now, neither complete bacteriophage reproduction nor stable integration of whole phage genomes in higher eucaryotic cells has been described. However, the induction of plant tumors by the Ti plasmid of *Agrobacterium tumefaciens* represents a natural case of stable

incorporation of bacterial genes in eucaryotic cells (273, 428, 540).

After infection of *Hordeum vulgare* protoplasts by T3, the phage genome is expressed to a limited extent (54). Regarding the fate of T7 DNA after experimental introduction into animal cells, there are conflicting results: Leavitt et al. (266) demonstrated the uptake of T7 DNA into the nucleus of Syrian hamster embryonic cells and its persistence throughout several cell division cycles; Kao et al. (215), in contrast, observed rapid digestion of T7 DNA after its entry into human skin fibroblasts. These and other results (see reference 139) invite the speculation that, analogous to the situation in bacteria (11, 37, 504), restriction systems with the ability of degrading foreign DNA also exist in eucaryotic cells (47, 416). Maybe these restriction systems are not yet fully expressed in embryonic, compared with adult, cells.

Richter et al. (389) detected a limited transcription and translation of T3 and T7 DNA in a cell-free system of yeast mitochondria. That phage DNA expression is not confined to the procaryote-like mitochondrial system was demonstrated by Anderson et al. (9), who found that T3 and T7 early mRNA's are translated on the 80S ribosomes of a mammalian cell-free cytoplasmic system. The translation efficiency of gene 0.3 mRNA is higher when using RNase III-processed mRNA than with polycistronic RNA. Capping of mRNA (modification of the 5′ end which facilitates translation of eucaryotic mRNA) seems not to be necessary for phage mRNA translation (9).

The purpose of these studies is twofold: first, they may help to illuminate the differences and correspondences in the molecular biology of pro- and eucaryotes, and, second, these studies could be of practical value for future experimental gene transfer with recombinant DNA from proto eucaryotes.

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